National Research Council Canada
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Plant Biotechnology Institute

Annual Report 1988-1989

Canada

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The National Research Council in Biotechnology

Canada's National Research Council (NRC) is a crown-owned corporation created to promote, assist and perform research of economic and social benefit to the nation. NRC operates more than a dozen national laboratories along with programs for scientific-technical information and industrial research assistance.

Since 1924 the Council has maintained a strong program in biosciences, and in 1983 was designated by the Canadian Government to lead the national effort in biotechnology development. To this end the programs of the Division of Biological Sciences (Ottawa), the Biotechnology Research Institute (Montreal) and the Plant Biotechnology Institute (Saskatoon) have been brought together in a unified Biotechnology Program.

In this program the Plant Biotechnology Institute has the mission of delivering new, exploitable biological and biochemical methods for the control and genetic alteration of plant development, especially at the cellular and molecular levels.

This mission reflects NRC's purpose under the National Research Council Act, to "promote research (i)...on the utilization of the natural resources of Canada; (ii) with the object of...discovering processes and methods that may promote the expansion of existing or the development of new industries; (iii)...to improve conditions in agriculture".

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Cover photo : Conifer tree seedling regenerated from cell cultures.

1. Highlights of the Year 1988/89

The Plant Biotechnology Institute (PBI) in Saskatoon is one of the national centres for advanced research and development operated in Canada by the National Research Council (NRC). PBI's main purpose is creation of key new plant biotechnology for use in Canadian economic development. The Institute assists research in other Canadian organizations and disseminates information about advances in the field. This report details the Institute's activities and accomplishments during the fiscal year April 1988 through March 1989.

Plant genetic engineering is the focus of the Institute. Four key enabling techniques for this appear throughout this report : the isolation and characterization of genes governing useful traits; the insertion of foreign genes into plant cells to produce "transformed" germplasm; the regeneration of whole plants from plant cell cultures (transformed or not); and the regulation and control of the action of genes in plants. The differing challenges of various types of plants are taken up in the different projects at PBI, but the Institute's program has now been completely unified around key methodologies, not commodities. For example, the Institute carries out no field breeding programs based on new germplasms from its laboratories; for "product development" we work with industrial partners. By this approach we have been able to enter valuable collaborations with extramural groups, and to complement the work of organizations in both the public and private sectors whose interests are in commodity development.

During the year the Institute housed, at any given time, about 95 NRC researchers and 24 supporting program staff. The Institute also maintained and assisted 86 on-site guest researchers from a wide variety of Canadian and foreign organizations. These organizations invested slightly more than $1 million at PBI to support their activities here, and NRC provided an operating budget (exclusive of salaries) of $4.3 million. Thus the Institute has truly become a multi-user national centre. Moreover, increases in guest activities seem likely to continue for the next few years.

Generation of brand-new technology occurs in the laboratories, and this is where PBI continues to invest more than three-quarters of its budget. During 1988/89, some discoveries of major significance came from project work at the Institute. These include :

- Construction of a totally synthetic gene conferring freezing protection in cells via its gene product. Some plant cell cultures were successfully transformed using this gene. We believe that the way is now open to genetic engineering of crop plants for frost resistance. (See Chapter 3);

- Transformation of conifer tree material using various reporter genes, and observation of gene expression in transformed spruce and pine. Improvements were also made in cell-to-seedling regeneration techniques,

bringing transgenic forest plants an additional step closer to reality. (See Chapter 3);

- Demonstration that G-banding techniques can be used in plant chromosome analysis. Formerly it had been believed that the valuable technique could not be applied to plant systems. (Chapter 3);

- Development of new hyper-promoters for plant genes has permitted high levels of expression to be achieved in transformed Brassica and other genera. In adaptation with our novel fused genes, these regulatory elements provide powerful tools for improved measurement of gene activities. (See Chapter 4);

- Creation and extensive testing of novel abscisic-acid analog molecules regulationg germination and related plant processes in a wide array of plants. The commercialization of these materials, now begun, represents development of a whole new class of useful agri-chemicals. (Chapter 5).

Collaborations were very numerous in 1988/89. Projects with other NRC biotechnology centres as partners continued to be assisted by NRC Biotechnology Comprehensive Programs funds. Some of the many PBI-industry joint research ventures were supported through the Contributions program while others had the participation of the Industrial Research Assistance Program (IRAP). An encouraging number of research ventures obtained strong direct funding from the industrial partner, especially where use of the Institute's unique facilities was an important factor in the companies' choice of PBI as a partner.

Two companies maintained sizeable incubator-type laboratory groups in the Institute's premises throughout the year. Philom Bios Inc. had seven researchers, and Vipont Research Laboratories Inc. nine, at year-end. The Saskatchewan Wheat Pool increased the size of its group during the period, and more than a dozen other companies placed researchers in PBI laboratories this year.

Our excellent facilities, the increasing store of licensable protected PBI technology, the growing effectiveness of the NRC's machinery for doing business, and the strong welcome extended to R&D partners brought all aspects of our business to new heights in 1988/89. This growth in industry-Institute connections has been particularly gratifying against the background of the difficult times being experienced by most plant biotechnology based companies in Canada. Chapter 6 details the results of the year's business program.

PBI-university collaboration was also very extensive throughout the year. By year-end, fifteen of the Institute's Research Officers held adjunct appointments as professors at various Canadian universities, and twelve extramural scientists had accepted appointments as Affiliated Scientists of PBI. Many university students and faculty carried out research in the Institute's laboratories, and some Institute scientists were posted into university laboratories for up to twelve months. Under the aegis of the Plant Biotechnology Fund more than half a million dollars worth of PBI's project research was contracted out competitively, this year the entire sum going to universities in Canada.

Like our sister NRC biotechnology centres in Ottawa and Montreal, we invested money in improving our on-site facilities and services for clients. A gene services laboratory providing automated DNA sequencing and oligonucleotide synthesis was completed, and through renovations we added another working laboratory unit for industrial guests. Provision for greenhouse growth and assessment of transgenic plants could not be made within our current building; during 1988 we therefore entered into agreements with the Saskatchewan Economic Development Corporation to obtain space in a large, new high-specification greenhouse complex scheduled to open in the autumn of 1989. This space will be accessible by our collaborators and research partners and will

provide a stronger base for technology transfer from PBI to the plant breeding community.

Success in producing useful new technologies depends ultimately upon the excellence of research personnel. In the period 1985 to 1988, six PBI researchers were among the top twenty most cited Canadian authors in plant biotechnology, and the Plant Biotechnology Institute topped the list of public Canadian laboratories in patented, licensed new technology. During 1988/89 three top-notch young scientists established themselves as new NRC Research Officers at PBI : Dr. Edward Tsang as a cell biologist in the conifer group, Dr. Janet Taylor as a molecular biologist in the Brassica area, and Dr . Ravindra Chibbar as a molecular biologist in monocot transformation work. These appointments completed the roster of continuing staff scientists within the present sizing of the Institute.

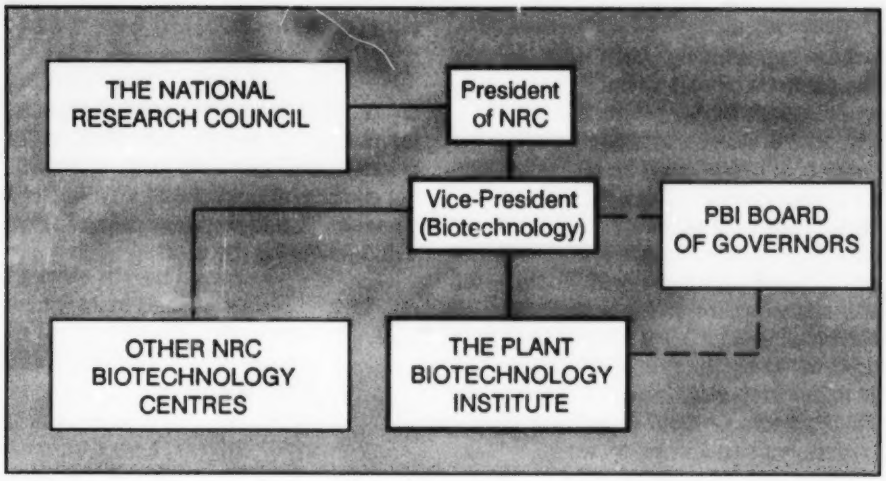
Through careful selection of research areas, effective organization and close personal teamwork, the Institute continued to provide scientific and technical leadership and service for the development of plant biotechnology in Canada. This report describes some of PBI's activities during the year.

Warren Steck

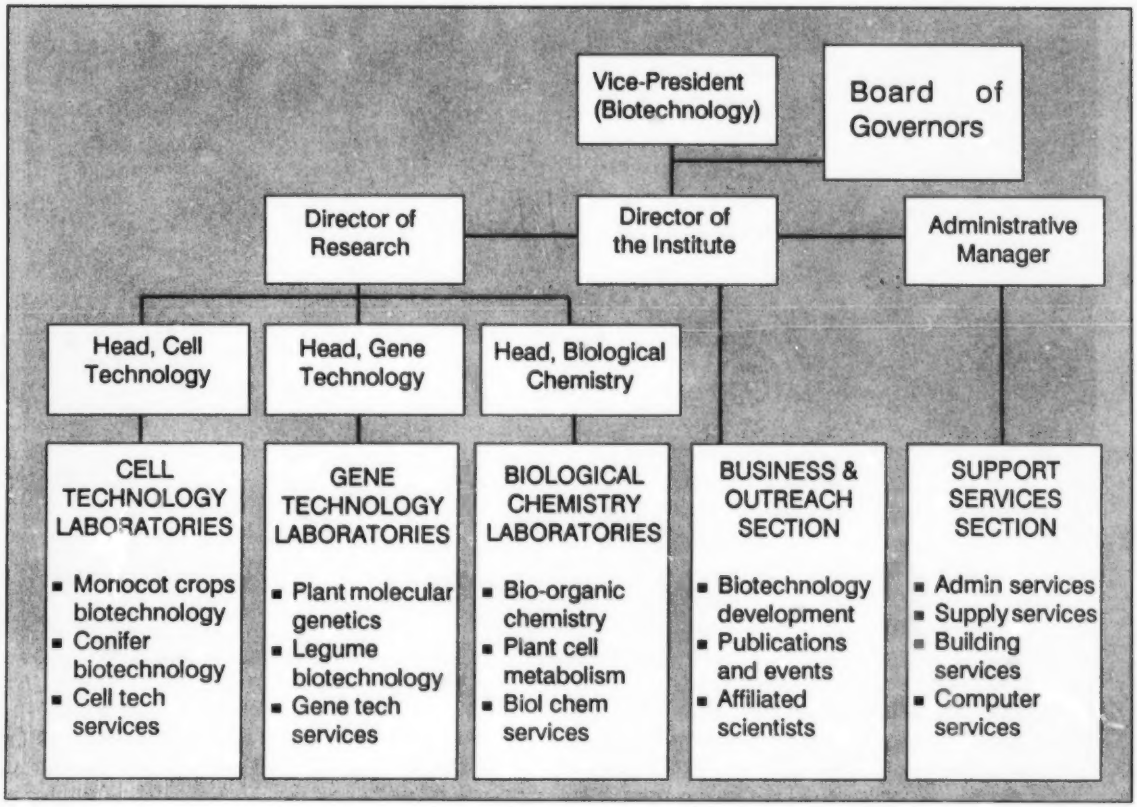
Warren Steck
Director of the Institute

Aerial view of the NRC's Plant Biotechnology Institute in Saskatoon.

PBI's Management by NRC



Organization of the Plant Biotechnology Institute in 1988/89

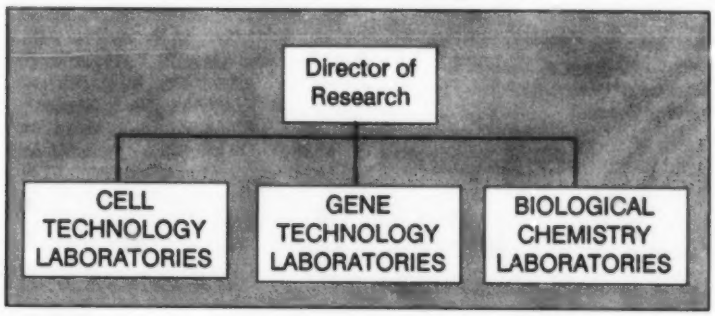


2. Organization and Personnel

2.1 Organization

The Plant Biotechnology Institute in Saskatoon is one of three divisions of the National Research Council which underpin the Council's biotechnology program. The lines by which NRC manages the Institute are indicated in the smaller diagram on the opposite page, with the Institute's internal organization shown in the larger diagram. Three of the "sections" of the Institute are devoted to laboratory research to create new biotechnology; a fourth section is dedicated to transfer of new technologies to users; and the fifth provides ongoing support for the others.

The Research Laboratories



2.2 The Board of Governors

The Institute's Board was established in 1987 under NRC's policy of having its national laboratories advised and overseen by the sectors they serve. The Board's terms include provision of strategic advice examination of the annual Plan of the Institute, and comment on resource requirements for the program.

Chairman of the Board: **Dr Maurice BROSSARD**, Senior Vice-President (Laboratories) and Vice-President (Biotechnology), National Research Council of Canada.

Other members, with their titles as of 31 December 1988, are:

Dr. Guy BELLEMARE, Professor, Faculty of Science, Laval University, (Sainte-Foy, Québec)
Dr. Wallace BEVERSDORF, Professor and Chairman, Department of Crop Science, University of Guelph (Guelph, Ontario)
Dr. Ian De La ROCHE, Assistant Deputy Minister Western Economic Diversification Canada (Saskatoon, Saskatchewan)
Dr. Leo KRISTJANSON, President, University of Saskatchewan (Saskatoon, Saskatchewan)
Dr. Maurice LALONDE, Professor of Biology, Laval University (Sainte-Foy, Québec)
Dr. Kelvin OGILVIE, Vice-President (Academic), Acadia University (Wolfville, Nova Scotia)
Dr. Warren STECK, Director, Plant Biotechnology Institute (NRC)
Mr. Ted TURNER, President, Prairie Pools Inc. (Regina, Saskatchewan)
Mr. Gordon WELLS, Chief Executive Officer, Biotechnica Canada (Calgary, Alberta)
Dr. John WILLIAMS, Professor of Botany, University of Toronto (Toronto, Ontario)

The Board met twice during the fiscal year. Both meetings were held in Saskatoon.

2.3 Staff, Affiliates and Guests

An asterisk (*) indicates a limited-term staff appointment. Some staff were not present for the entire year.

DIRECTOR: Warren F. STECK, B.Eng. (McGill); Ph.D. (Saskatchewan)

ASSISTANT DIRECTOR Friedrich CONSTABEL, Dr. Res. Nat. (Göttingen), Dr. Habil. (Bonn)

ADMINISTRATIVE MANAGER: Joan L. YORKE

PRINCIPAL RESEARCH OFFICERS:
Wolfgang G.W. KURZ, Ph.D. (Vienna)
Edward W. UNDERHILL, B.S.P., M.Sc. (Saskatchewan), Ph.D. (Rhode Island)

SENIOR RESEARCH OFFICERS:
David I. DUNSTAN, B.Sc., Ph.D. (NE London Polytechnic)
Kuo-Nan KAO, B.S. (Chung-Hsing, China), M.S.A. (Guelph), Ph.D. (Saskatchewan)
Kutty K. KARTHA, B.Sc. (Saugar), M.Sc. (Jawaharlal), Ph.D. (New Delhi)
Samuel L. MacKENZIE, B.Sc., Ph.D. (Edinburgh)
John D. MAHON, B.Sc.(Western Ontario) M.Sc., Ph.D. (Queen's)
George P. SLATER, B.Sc. (Aberdeen), M.Sc. (Saskatchewan), Ph.D. (Queen's, Belfast)

ASSOCIATE RESEARCH OFFICERS:
Suzanne R. ABRAMS, B.Sc. (Carleton), Ph.D. (Dalhousie)
John J. BALSEVICH, B.Sc., M.Sc., Ph.D. (B.C)
Ravindra N. CHIBBAR, B.Sc., M.Sc. (Meerrut), Ph.D. (Panjab), Ph.D. (Western Ontario)
William L. CROSBY, B.Sc. (British Columbia), Ph.D. (Edinburgh)
Adrian J. CUTLER, B.Sc. (Wales), Ph.D. (Florida State)
Vincenzo DELUCA, B.Sc. (Concordia), M.Sc.(Queen's), Ph.D. (Western Ontario)
Fawzy GEORGES, M.Sc. (Brock), Ph.D. (Ottawa)
Jan W.D. GROOTWASSINK, B.Sc., M.Sc. (Wageningen), Ph.D. (Calgary)
Sean M. HEMMINGSEN, B.Sc. (British Columbia), Ph.D. (Queen's)
Shaun L.A. HOBBS, M.A. (Cambridge), B.A., D. Phil. (Oxford)
Martin S. LAPP, B.Sc., M.Sc. (York), Ph.D.(Alta.)
Mstaslaw MAZUREK, B.S.A. (Saskatchewan)
Louise M. NELSON, B.Sc. (Western Ontario), Ph.D. (Calgary)
Lawrence E. PELCHER, B.S. (Aquinas), M.S., Ph.D. (Michigan State)
Mohammed SALEEM, B.Sc., M.Sc. (Pakistan), M.Sc. (Laurentian), Ph.D. (Western Ontario)
Gopalan SELVARAJ, B.Sc. (Bangalore); M.Sc., Ph.D. (Carleton)
Robert T. TYLER, B.S.A., Ph.D. (Saskatchewan)

ASSISTANT RESEARCH OFFICERS:
Janet L. TAYLOR, B.Sc. (Iowa State), Ph.D. (Washington State)
Edward W.T. TSANG, B.Sc., M.Sc., Ph.D. (Saskatchewan)

RESEARCH COUNCIL OFFICERS:
J. Sylvan KATZ*, B.Sc. (Saskatchewan)
T. Martin MALLARD

NSERC FELLOWS FUNDED BY PBI:
Faouzi BEKKAOUI*, B.Sc. (Berkane), M.Sc.(Tours, France), Ph.D. (Paris)
Lesh KLIMCZAK*, M.Sc. (Biology), Ph.D. (Würzburg)
Lloyd A.K. NELSON*, B.Sc. (U.S.T.), Ph.D.(Alberta)

RESEARCH ASSOCIATES:
Luc ADAM*, B.Sc., Ph.D. (Laval)
Sylvain ADAM*, M.Sc. (Orsay), D.E.A., Ph.D. (Paris)
Joseph G. BOOTHE*, B.Sc., Ph.D.(Western Ontario)
Christopher P. CHANWAY*, B.Sc., B.S.A. (Manitoba), M.Sc., Ph.D. (British Columbia)
Supa CHAVADEJ*, B.Sc., M.Eng. (Chulalongkorn), Dr. Res. Nat. (Heidelberg)
Lynn CLONEY*, B.Sc., Ph.D. (New Brunswick)
Allen GOOD*, B.Sc. (Trent), M.Sc.(Windsor), Ph.D. (Ottawa)
Atta HUSSAIN*, B.Sc. (Iraq), M.Sc. (Birmingham), Ph.D. (Aston)
Jennie JACKSON*, B.Sc.(Hons) (St. Andrews), Ph.D. (Wales)
Jinesh C. JAIN*, B.Sc. , M.Sc. (Rajasthan), Ph.D. (Jodhpur)
Edward J. KENDALL*, B.Sc., M.Sc. (Dalhousie), Ph.D. (Guelph)
Marie-Françoise LeGAL*, D.E.A., Ph.D. (Nantes, France)
Remi MARTEL*, B.Sc., M.Sc., Ph.D. (Laval)
Brenda McAFEE*, B.Sc., M.Sc., (Acadia), Ph.D. (Laval)
W. Jay NEWSTED*, B.Sc., M.Sc., Ph.D. (Western Ontario)
Nancy L. PAIVA*, B.Sc.(Harvey Mudd), PhD. (M.I.T.)
M. Soledade C. PEDRAS, B.Sc., M.Sc. (Porto), Ph.D. (Alberta)
Gregory PENNER*, B.S.A., M.Sc., (Manitoba), Ph.D. (Saskatchewan)
Gilles PILATE*, B. Ed., M.Sc., Ph.D. (Paris)
Javed QURESHI*, B.Sc., M.Sc. (Karachi), Ph.D. (Liverpool)
Datla S. RAJU*, B.Sc., M.Sc., Ph.D. (Andhra)
Hans REMPEL, B.Sc., Ph.D. (California State)
Ingebord ROEWER*, M.Sc., Ph.D. (Muenster)
David TAYLOR*, B.Sc., Ph.D. (Carleton)
Lawrence W. THOMSON*, B.Sc., M.Sc., Ph.D. (Alberta)
Randall J. WESELAKE*, B.Sc.,M.Sc., Ph.D. (Manitoba)

VISITING RESEARCH OFFICER
Nikolaus WEBER*, B.Sc. (Mainz), M.Sc., Ph.D. (Heidelberg)

LABORATORY TECHNICAL OFFICERS
Maureen ANDERSON
David BALISKI
Carla BARBER, B.Sc. (Saskatchewan)
Dennis BARTON, B.Sc. (Saskatchewan)
Mary BECKIE, B.Sc. (Saskatchewan)
Colleen BENNETT*, B.S.T. (K.I.A.A.S.)
Terry BETHUNE, B.Sc. (Saskatchewan)
Greg BISHOP, B.Sc. (Saskatchewan)
Cheryl BOCK, B.Sc. (Saskatchewan)
Ira BORGMANN, B.Sc., M.Sc., (British Columbia)
Karen CASWELL, B.S.A., M.Sc. (Saskatchewan)
K. Brock CHATSON, C.R.T. (S.A.I.T.)
Maxine COFFEY, B.L.T. (Regina)
Janet CONDIE, B.Sc. (Queen's)
Catherine DELONG, B.S.A., B.Sc. (Saskatchewan)
John DYCK*
Don DYMOND
Mike GIBLIN
Joseph HAMMERLINDL
Rozina HIRJI, B.Sc. (Makerere)
Lawrence HOGGE
Les HOFFMAN
David HORN
Connie KNAPP
Diana KNEESHAW, B.S.A. (Saskatchewan)
Allen KOLENOVSKY, B.Sc. (Saskatchewan)
Nick LEUNG, B.Sc. (Saskatchewan)
Mary LOEWEN, B.S.A. (Saskatchewan)
Cliff MALLARD
John MAGUS, B.Sc.(Saskatchewan)
Jana MALINEK, B.Sc., M.Sc. (Palacky)
Morris MICHAYLUK
Melanie OLSON*, B.Sc. (Saskatchewan)
Doug OLSON, B.S.T. (K.I.A.A.S.)
Keith PAHL, B.S.T. (K.I.A.A.S.)
Barry PANCHUK
Bob PAPISH, B.Sc. (Saskatchewan)
Sandra POLVI, B.S.T. (K.I.A.A.S.)
Darwin REED, B.Sc. (Saskatchewan)
Don SCHWAB, B.S.T. (K.I.A.A.S.)
Angela SHAW, B.Sc. (Saskatchewan)
Jerry SHYLUK
Lee STEINHAUER, B.A. (Saskatchewan)
Don TENASCHUK, B.Sc. (Saskatchewan)
Maureen WOOD*, B.S.T. (K.I.A.A.S.)

ADMINISTRATIVE SERVICES:
Cheryl BITZ
Rosemarie GALLAYS
Dawn LARSON
Kathryn SEABROOK
Carol WALKER
Katherine WILSON

SUPPLY SERVICES:
Olga COATES
Jill HORN
Irene HOWE
Terry MAZURKEVICH
Jim McHARG
Bill WILEY

RESEARCH ILLUSTRATION:
Ed KNAPP

GENERAL SERVICES:
Bill BRUMWELL
Jim DRINKWATER*
Armand FENDELET
Harry HOLFELD
Rick McDONALD
George REYNOLDS
Wayne STANKEWICH

PUBLICATIONS AND EVENTS:
Lorraine BLASHILL
Carolyn HELLQUIST*

SUMMER STUDENTS 1988
Katherine BARRETT*
Helene BOURASSA*
Michael CHESTER*
Kenneth HAMPEL*
Michael LEVY*
David NEUSTAEDTER*
Michelle PEEL *
Mark PILON *
Barry PITTENDRIGH *
Ruth POWER *
Dana SCHROEDER *

AFFILIATED SCIENTISTS

Helmut BERTRAND, B.A., M.Sc., Ph.D. (Kansas), Professor of Microbiology, University of Guelph

Normand BRISSON, B.Sc., M.Sc. (Montreal), Ph.D. (McGill) Professor of Biochemistry, University of Montreal

Keith DOWNEY, B.Sc., M.Sc., (Saskatchewan), Ph.D. (Cornell), Head, Oilseeds Section, Agriculture Canada Research Station,(Saskatoon, Sask.)

Lawrence FOWKE, Ph.D. (Carleton), Professor of Biology, University of Saskatchewan (Saskatoon, Sask.)

Bryan L. HARVEY, B.S.A., M.Sc., (Saskatchewan), Ph.D. (California),Professor and Head of Crop Science and Plant Ecology, University of Saskatchewan; Director of the Sask-atchewan Crop Development Center (Saskatoon, Sask.)

George HAUGHN, B.Sc. (Dalhousie), Ph.D. (Cornell) Professor of Biology, University of Saskatchewan

Wilfred A. KELLER, B. Sc., Ph.D. (Saskatchewan), Head, Genetic Engineering Section, Plant Research Centre, Agriculture Canada (Ottawa,Ont.)

Dr. Brian MIKI, B.Sc.(Toronto), Ph.D. (Carlton), Plant Research Centre, Agriculture Canada, (Ottawa, Ont.)

Maurice MOLONEY, B.Sc.(London), Ph.D. (Leicester) Professor of Biology, University of Calgary

Ann OAKS, B.A., M.A. (Toronto), Ph.D.(Saskatchewan) Professor of Biology, Guelph University

Trevor L. THORPE, B.Sc. (Allahabad, India), B.Sc., Ph.D. (California) Professor of Biology, University of Calgary (Calgary, Alberta)

G. H. Neil TOWERS, B.Sc., M.Sc. (McGill), Ph.D. (Cornell) Professor of Botany, University of British Columbia (Vancouver, B.C.)

GUEST RESEARCHERS AT THE INSTITUTE, 1988/89

Dr. Garth Abrams, Crop Development Centre
Dr. Jesus Alvarez, National Research Council of Spain
Ms Alison Baillie, University of Saskatchewan
Dr. K. Balasubramanian, University of Saskatchewan
Dr. Vipan K. Bansal, Agriculture Canada
Ms M. Beckie, professional services
Dr. Sant Bhojwani, University of Delhi (India)
Ms. Lesley A. Boyd, University of Saskatchewan
Mr. Robin Browne, University of Saskatchewan
Mr. H. Cheng, Genetic Institute of Jiangsu Academy of Agricultural Sciences, Nanjing (China)
Dr. Normand Chevrier, Université du Québec (Montréal)
Ms. Cheryl Craig, University of Saskatchewan
Mr. Jim Cunningham, Philom Bios Inc.
Dr. Alain P. David, Université de Bordeaux (France)
Dr. Hélène David, Université de Bordeaux (France)
Dr. Laurence Davin, Université de Paris (France)
Dr. Peter S.S. Dawson, Biotechnology Research Institute
Ms. Monique Dethier, Université Louvain la Neuve (Belgium)
Mr. John Dyck, professional services
Ms. Patricia Englund, Vipont Research Laboratories Inc.
Mr. Delwin Epp, Vipont Pharmaceuticals Inc.
Ms. Laurie Friesen, Vipont Research Laboratories Inc.
Dr. Basu V. Gantotti, Philom Bios Inc.
Dr. Kenneth Giles, Vipont Research Laboratories Inc.
Dr. Larry Gusta, Crop Development Centre
Ms. Cheryl Hampson, University of Saskatchewan
Mr. Ronald Harkrader, Vipont Research Laboratories Inc.
Dr. George Haughn, University of Saskatchewan
Mr. Dwayne Hegedus, University of Saskatchewan
Dr. Harriet Hemmingsen, Oxford University (U.K.)
Ms. Carol Hill, Vipont Research Laboratories Inc.
Dr. Sjoichi Ichihashi, Aichi University (Japan)
Mr. Ravinder Jain, University of Saskatchewan
Mr. Blair Jarvis, University of Saskatchewan
Ms. Grace Johnson, Saskatchewan Wheat Pool
Mr. Raman Kapur, University of Saskatchewan
Ms. Susan Kikcio, University of Calgary
Dr. John King, University of Saskatchewan
Dr. Lesh Klimczak, NSERC Fellow
Ms. Kristina Komendant, Philom Bios Inc.
Ms. Kathy Kuiack, Philom Bios Inc.
Dr. Ljerka Kunst, University of Saskatchewan
Mr. Eric Lainé, Université de Toulouse (France)
Dr. Nancy Lamb, Crop Development Centre
Ms. Debbie Lashta, Philom Bios Inc.
Ms. Monika MacHutchon, University of Saskatchewan
Ms. Sourindra N. Maiti, University of Saskatchewan
Dr. Chai Mak, University of Singapore
Mr. Braden Manns, University of Saskatchewan
Ms. M. Luisa Marin, CIAT (Colombia)
Ms. Marilyn Martin, University of Saskatchewan
Ms. Linda McGregor, Saskatchewan Wheat Pool
Ms. Maria Moore, Weyerhaeuser Canada Ltd.
Mr. N. Nehra, University of Saskatchewan
Dr. Lloyd A.K. Nelson, NSERC Fellow
Mr. Y. Niu, University of Saskatchewan
Ms. Debra Novakovski, Vipont Research Laboratories Inc.
Ms. Laurel O'Connor, Philom Bios Inc.
Mr. Brian Orshinsky, Saskatachewan Wheat Pool
Dr. Jong Park, Vipont Research Laboratories Inc.
Dr. Kenneth Pivnick, Agriculture Canada
Ms Patricia Polowick, professional services
Ms. Ruth Power, University of Saskatchewan
Mr. Martin Reaney, University of Saskatchewan
Mr. Kevin Rozwadowski, University of Saskatchewan
Ms. Susan Scutchings, University of Saskatchewan
Ms. Debbie Sommer, University of Nebraska
Dr. David Songstad, Vipont Research Laboratories Inc.
Ms. Sharon Sprout, University of Saskatchewan
Mr. Dan Stewart, Blueberry Acres Ltd.
Mr. Jim Sutherland, professional services
Mr. Stephen Tallevi, Biomèga Ltée
Mr. Tom Tautorus, Stange Canada Inc.
Mr. Mike Thompson, University of Calgary
Dr. Leena Toivonen, Finnish Academy of Science
Dr. H.-C. Wang, University of Saskatchewan
Mr. Hong Wang, University of Saskatchewan
Mr. Roger Warren, University of Saskatchewan
Mr. Michael Whiting, University of Saskatchewan
Dr. David Williams, University of Saskatchewan
Ms. Linda Wong, University of Saskatchewan
Mr. David Woodske, Philom Bios Inc.
Ms. Adrienne Woytowich, University of Saskatchewan
Mr. Guohai Wu, University of Saskatchewan
Ms. Melinda Yiu, University of Saskatchewan
Mr. Lu Zhang, University of Saskatchewan

3. Cell Technology Laboratory

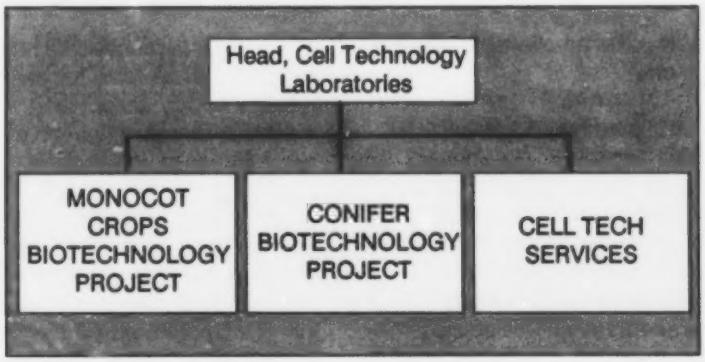
Section Head: K. K. Kartha

3.1 Overview

The Cell Technology Section grew out of PBI's pioneering work in the 1960s and 70s on the manipulation of plant cells in artifical culture. The Institute continues to provide the best scientific expertise and facilities available in this field for Canadian industries and research organizations.

During 1988/89 one new laboratory unit was acquired, bringing the total number of working units to eleven : three for conifer studies, five for monocot systems, two for molecular biochemistry and one for cryopreservation. We achieved a significant advance in transformation facilities with the installation of a micro-ballistic particle gun in February 1988.

Two large and important projects were fielded by the Section during the year. The **crop biotechnology** project aims at the development of molecular and cell techniques for genetic manipulation of cereal grains and has cold tolerance as a major theme. The **conifer biotechnology** project is developing techniques for the micropropagation and genetic transformation of such economically important trees as spruce and pines. The general structure of the Section is shown schematically in the diagram below.



3.2 Crop Biotechnology Project

3.2.1 Project Personnel

Research Officers: K.K. Kartha (Leader), A. Cutler, R. Chibbar, F. Georges, K.N. Kao, M. Saleem

Research Associates: J. Boothe, E. Kendall, J. Newsted, J. Qureshi

Technical Officers: K. Caswell, M.A. Coffey, D. Horn, N.L. Leung, M.K. Loewen, C.S. Mallard, M. Michayluk, K. Pahl, S. Polvi, J.P. Shyluk, L. Steinhauer

Guest Researchers: A. Baillie, M. Beckie, N. Chevrier, J. Dyck (casual), R. Kapu, M.L. Marin, L. McGregor, M.McHutchon, N. Nehra, Y. Niu, B. Orshinsky, P. Polowick (casual), H. Wang, H.C. Wang

Summer Student Assistants (1988): K. Hampel, D. Schroeder, M. Peel

3.2.2 Introduction

The overall objective of the project is the genetic improvement of selected Canadian monocot crop plants (wheat, barley) through advanced cell culture, cell selection and genetic engineering technologies. The specific objectives are the creation of monocot plants with improved agronomic traits such as resistance to cold and herbicides, along with some parallel applications in sunflower and canola.

The Crop Biotechnology Project was formed, within the Cell Technology Laboratory, in 1984. After extensive consultations with various user sectors and following establishment of joint venture arrangements with universities and the private sector (Saskatchewan Wheat Pool), the group decided to place research emphasis on cereal crops (wheat and barley). This decision was made at a time when there existed, in Canada, almost no research activity in cereal biotechnology despite the importance of cereal crops for Canada.

This lack of research activity is partly attributable to the extreme recalcitrance of *in vitro* cultures of cereals. No systematic and detailed study had been carried out anywhere to assess the tissue culture responses leading to predictable whole plant regeneration with Canadian cultivars. This meant that in order to exploit the full potential of biotechnology, the Project had to develop appropriate *in vitro* cereal culture techniques.

We believed that with the very considerable cell culture expertise resident within the laboratory, such techniques could be developed rapidly. Accordingly, a team effort was put in place in 1986 and developed around the objectives mentioned above.

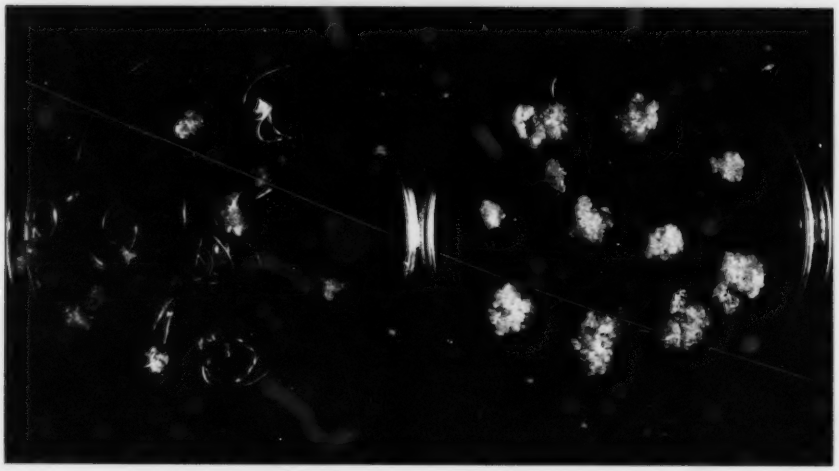
3.2.3 Progress in 1988/89

In vitro cell systems with predictable plant regeneration capabilities have been developed with members of the *Solanaceae* (potato) and *Brassicae* (mustard) families, but progress made with the *Gramineae* (grass family) to which our target crops (wheat, barley) belong,was slow until 1988. During 1988/89 we made rapid progress in this and related areas of plant generation, selection and assessment.

3.2.3.1 Wheat

We demonstrated high efficiency plant regeneration systems for over 10 diverse genotypes of spring wheat using 10-to 14-day-old immature embryos. Induction and maintenance of regeneration in wheat callus was dependent upon judicious *in vitro* culture regimes. A callus line induced after mutagen application of immature embryos has retained high plant regeneration capability even after three years of subculture, showing no loss of morphogenetic capability. The developmental stage of wheat embryos at the time of initiation of culture is crucial.

Fourteen-day and older embryos, when cultured, undergo direct germination, thus limiting the choice of explant material. To expand



(Left) Embryos cultured with MeABA. Germination of embryonic axis, no scutellar callus. (Right) Embryos cultured with MeABA (1mg/L) germination is inhibited,scutellar proliferation and somatic embryogenesis. J. Qureshi photo.

the range and choice of explant material, zygotic embryos from 10 spring wheat genotypes were tested for embryogenic callus induction in the presence of externally supplied (±) abscisic acid (ABA) and two of its analogs, methyl abscisate and methyl epoxy-beta-ionylideneacetate. ABA and the analogs suppressed precocious germination of cultured late-stage embryos and promoted embryogenic callus induction, so that many more plants were regenerated from callus induced in the presence of ABA and ABA-analogs.

While we have developed excellent regeneration systems with 10 different genotypes of spring wheat, one other genotype exhibited a very low rate of regeneration. This line provided us with an opportunity to assess the genetic basis of plant regeneration. Through collaboration with the Saskatchewan Wheat Pool, several crosses were made between high responding and low responding genotypes and subsequent generations were tested for regeneration potential. This study revealed that regenerability is genetically controlled, is heritable, and can be incorporated into genotypes of interest which are "recalcitrants". Thus, our efforts to develop highly efficient plant regeneration systems from somatic tissues of wheat have been successful. Our present cultures offer an endless supply of morphogenic material for gene transfer through microprojectile bombardment.

Wheat Haploidy - Haploids are extremely valuable material for plant breeders, for double haploids are pure lines which can be directly used in breeding programs. Conventional production of pure lines requires several years of selection and back-crossing. Under an IRAP-assisted project with Saskatchewan Wheat Pool, work has been initiated to develop haploidy systems for several classes of wheat and for use in breeding for novel traits. This research has been very successful, with doubled dihaploids already undergoing field testing and evaluation.

3.2.3.2 Barley

Barley Regenerants- We optimized the production of embryogenic callus and regenerant plants from immature embryos of several Canadian barley varieties. "Ellice" produced the greatest number of regenerant plants; choice of auxin regime was important. Regenerant plants were grown to maturity. Seven hundred and sixty-four heads from four varieties were collected, and this seed was then grown out in the field under the terms of a collaborative research agreement between the Institute and the Saskatchewan Crop Development Cente. No reliable data were collected from this material in 1988 due to severe drought conditions; the seed

will be grown out again in 1989.

Barley Haploidy- We obtained green microspore-derived plants from any of the lines or varieties tested, up to 13 green plants from a single anther, with the average of all experiments being 1.5.

Cell Selection for Herbicide Tolerance in Barley - This work involved Ph.D. thesis research by Ms. Alison Baillie, in collaboration with Dr. B.G. Rossnagel of the Crop Development Center. Selection for tolerance to chlorsulfuron and trifluralin was carried out using *in vitro* selection strategies. Some tolerant cell lines were found and regenerated. Twenty-three such embryo- culture derived lines were evaluated in the greenhouse, and seed progeny of these plants were grown in soil containing up to 100 g active ingradient (ai)/ha chlorsufuron. (The field rate of this herbicide is 15 to 30 ai/ha.) Several selected lines showed more tolerance to chlorsulfuron than the parental stock.

Cereal Transformation - The genetic engineering of cereals has been hampered by the inefficacy of ordinary transformation methods.

We explored various strategies during the year: gamete mediated transformation, pollen tube pathway, development of protoplast system for electroporation, and lately microprojectile bombardment. By year-end some transformations had succeeded but no method of choice was yet apparent.

3.2.3.3 Biochemical and Molecular Aspects of Somatic Embryogenesis in cultured plant cells.

Esterases as markers of somatic embryogenesis - The main objective of this study is to develop biochemical markers for somatic embryogenesis. Since a specific pattern of esterases was earlier found in embryogenic cells we wanted to see if esterases are secreted in to the medium. Moreover, a unique esterase isozyme was also observed in the medium of a non-embryogenic cell line of the same species. This isozyme was purified to apparent homogeneity and antibodies were raised against this purified protein, in rabbits. However, these antibodies cross reacted with other extracellular proteins, so that a definitive result has not yet been obtained.

3.2.3.4 Molecular Aspects of Cold Tolerance in Wheat

Despite a great deal of research, cold acclimation in plants still is poorly understood. Being a polygenic trait, it offers especial challenge. Plants during low temperature acclimation or under desiccation stress accumulate high levels of carbohydrates, which have been implicated in the stress tolerance. We examined sucrose formation. Sucrose synthetase was purified from the crude wheat germ extracts usinf poly acrylamide gel followed by electroelution. The purified 95 Kd polypeptide, though denatured, formed sucrose from fructose and urdine diphosphate glucose. Development of probes against the synthetase is underway. To study the role of carbohydrates as cryoprotectants, levels of synthetase were examined in wheat exposed to low temperature. We extracted the total soluble proteins from winter and spring wheat seedlings subjected to prolonged low temperature (2°C) versus control plants grown at ambient temperatures. Results showed accumulation of 95 Kd synthetase subunit at 2°C in both winter and spring wheat shoots, these polypeptides being scarce or absent in the controls.

Cryoselection - In a previous Annual Report, we reported a novel cryoselection strategy for selecting cells which tolerate liquid nitrogen temperature (-196°C). During 1988/89,cryobiological and biochemical parameters were examined to characterize cryoselected wheat lines. In freezing tests carried out on various callus lines, small pieces of the compact regenerative calli were frozen without any treatment or cryoprotectant. Twelve controlled freezing rates were examined ranging from 0.1-30°C per minute to a terminal temperature of - 35°C followed by immersion in liquid nitrogen (-196°C). After rapid thawing, parts of all calli survived and in appropriate nutrient medium subsequently regenerated plants. Currently, these regenerants are being grown to multiply the seed stock for evaluation for freezing tolerance at the plant level.

Cold Affects Energy Metabolism - In a brief study the early effects of low non-freezing temperature stress and of oxygen deprivation were investigated in three wheat callus lines of graded hardiness. Only the hardiest line maintained adenosine triphosphate levels and mobilized energy reserves at low but non-freezing temperatures.

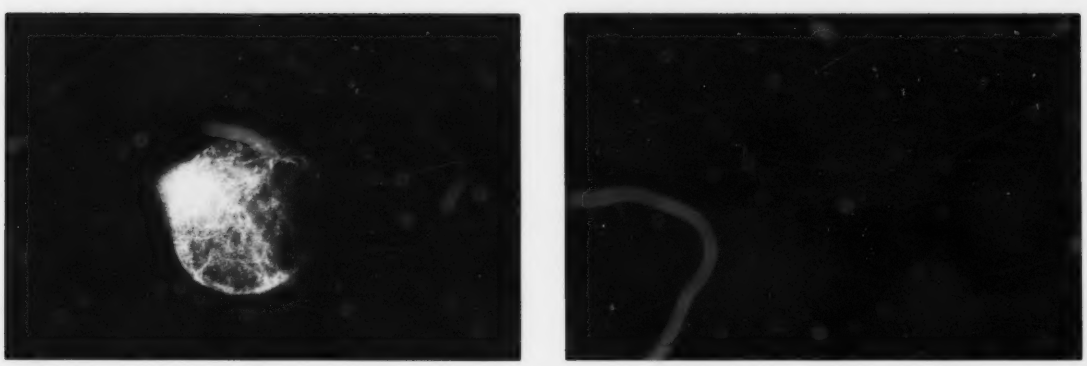
3.2.3.5 Use of Antifreeze Proteins to Improve Plant Cold Hardiness

In 1987 we obtained some evidence suggesting the possibility of engineering plants to produce anti-freeze proteins. In our project plans for 1988/89 our objectives included "...analysis of the effect of antifreeze protein on plant tissue..." and "Introduction of the flounder antifreeze gene into a protoplast system by electroporation...".

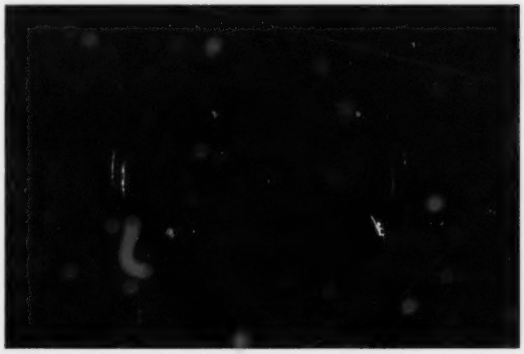
Protein Studies - The antifreeze protein experiments involved vacuum infiltration of antifreeze solutions into *Arabidopsis* leaves and measurement of equilibration-freezing temperatures by differential thermal analysis. The amount of protein introduced into leaf tissue was quantitated using 14C-labelled cytochrome-C as a standard. Under these conditions antifreeze protein lowered the spontaneous freezing temperature of *Arabidopsis* leaves by 4°C. The antifreeze protein promoted supercooling, a first line of defence against freezing damage, increasing supercooling in canola tissues by an average of 1.8°C. This is significant for late spring and early fall frosts, for reducing the lethal temperature from -1°C to -2°C would reduce the number of potentially lethal freezing events from 13 to 5.

Other experiments measured the cryoprotectant properties of the protein. Bromegrass suspension culture cells exposed to the protein contained a higher proportion of unfrozen water at freezing temperature than did controls. For example, under 30% of cellular fluids remained liquid in control cells at -7°C whereas cells exposed to 20 g/L of the antifreeze protein contained almost 50% liquid. This cryoprotectant action provides a second line of defense against freezing by reducing the extent of tissue disruption.

Antifreeze Protein Gene Expression - The objective was to develop a transient expression system for antifreeze protein gene



(Left) Microtubules in a freshly isolated plant protoplast. (Right) Nucleii of a plant protoplast.



Bacterial colonies harboring a switchable plasmid. Arrows highlight colonies carrying plasmids that had undergone the switching evrent successfully as indicated by the color reaction. F. Georges photo

constructs so that problems of expression in a heterologous host could be assessed without stably transformed plants. Other laboratories have failed (for unknown reasons), to obtain antifreeze gene expression in plants. We first synthesized a construct gene containing a codon selection appropriate to plant expression, and our second generation construct provided for stable *Agrobacterium*-mediated transformation. We used a CaMV 35S promoter.

Electroporation of our first construct into corn suspension culture protoplasts led to detectable chloramphenicol acetyl transferase (CAT) enzyme activity. Since pGCS1 codes for an antifreeze-CAT translational fusion product, this result implied antifreeze protein expression. We then confirmed antifreeze gene activity by finding its messenger RNA in transcription by probing RNA extracts electroporated protoplasts.

We next tried to demonstrate the antifreeze protein part (AFP) of the AFP-CAT the production of AFP protein in electroporated protoplasts. Since the CAT protein was produced in insufficient amounts to be detected by Western blotting we resorted to more sensitive analytical methods, but neither autoradiography nor or immunoprecipitation with anti-CAT provided evidence for the presence of AFP component of the fusion peptide. Other methods also failed, and we finally found that this protein is subject to very rapid degradation *in vivo*.

We hoped that the antifreeze protein would be secreted into the extracellular space, the location where it can function. Our plasmid included the fish leader or signal sequence responsible for secretion. However, this leader worked inefficiently in corn since only a small proportion of the total CAT activity was extracellular.

Technical officer Cliff Mallard monitors a gel electrophoresis experiment.

3.3 Conifer Biotechnology Project

3.3.1 Project Personnel

Research Officers: David Dunstan (project leader), Martin Lapp, Edward Tsang

Research Associates: Brenda McAfee (from December 1988), Gilles Pilate (from February 1989) Faouzi Bekkaoui (NSERC Fellow)

Technical Officers: Terry Bethune, Cheryl Bock, Krystyna Czechowicz, Jana Malinek

Summer Student: Marc Pilon

Guest Researchers: Robin Browne, Alain David, Hélène David, Susan Kikcio, Eric Lainé, Tom Tautorus

3.3.2 Introduction

The objective of the Project is the development of biotechnology enabling the rapid propagation and improvement of coniferous trees, for the provision of superior, elite, or genetically engineered specimens.

Canada's conifer forests of the future must produce high quality wood for lumber, pulp and paper applications. These trees must have the capacity for rapid growth and for resistance or tolerance to pests, disease and environmental extremes. It has proven difficult or impossible to fulfil these requirements by conventional methods, but biotechnology may be able to contribute to these aims. Our project aims to achieve its objectives by research in cell and tissue culture, and in molecular biology. Now in its fourth year, our group has established fruitful collaborations with researchers at the Universities of Calgary and Saskatchewan, at the Pacific Research Centre of Forestry Canada, and elsewhere.

Plant propagation by *in vitro* techniques allows genetically "elite" conifer germplasm to be multiplied rapidly into seedlings for field use. Whereas it may take twenty years for seed to form on a tree, cloned seedlings can be produced in the space of months. Present knowledge of micropropagation is poor and current processes inefficient and therefore costly. Our goal is to improve these processes for use in tree improvement programs.

3.3.3 Research Progress

3.3.3.1 Micropropagation

Work continued in micropropagation by organogenesis and embryogenesis. Our research with organogenesis of juvenile tissues from western white pine (*Pinus monticola*) led to improved shoot induction and elongation procedures. Elongated shoots have been rooted under conditions that remain to be optimized. Some of this material was transplanted, acclimatized, and sent to Forestry Canada (Pacific Research Centre) for evaluation.

Organogenesis studies with adolescent or adult tissues concentrated on Douglas fir (*Pseudotsuga menziesii*) because this well-developed culture system was a ready source of clonal shoots for rooting experimentation. Some derived roots were induced on shoots from an 18-year old tree. The effects of various media and of benzyl-adenine were examined in some detail, and we succeeded in finding conditions which greatly improved numbers of shoots obtained from each original embryo, and which allowed significant root formation.

Shoot induction in white spruce (*Picea glauca*) and *Pinus contorta* has been less fruitful. Analyses with cultured buds from 14-year-old grafted *Picea glauca* showed critical requirements for inorganic nitrogen compounds for shoot induction, forming the basis of ongoing experimentation. The sterilization problems previously noted with *Pinus contorta* have been minimized. Although we observed callus induction, no organogenesis, direct or indirect was obtained.

Scanning electron micrograph of a black spruce somatic embryo. Fawzy Georges photo.

Using somatic embryo suspension cultures we regenerated transplantable plantlets from material in culture for over 30 months. Somatic embryo suspensions of white and black spruce provided valuable tissue for protoplast isolation and subsequent regeneration.

3.3.3.2 Transformation

Genetic engineering of conifers by gene insertion is a potential method for creating new "elite" trees with traits not obtainable by sexual breeding, but little research has been reported on genetic transformation of conifers. Our goal in 1988/89 was to find transformation methods and to produce and study some transgenic cells.

White spruce protoplasts were transformed using electroporation, and transient expression of the new gene was observed. The beta-glucuronidase (GUS) marker proved unsuitable because of GUS activity in spruce cells, however chloramphenicol acyl transferase (CAT) markers gave satisfactory assays. Large effects of various cell lines and promoter were noted. Electroporation also gave transgenic black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*).

Somatic embryo suspensions have also proven valuable for experimentation in transformation. This was achieved transiently after electroporation of protoplasts of white and black spruce and of jack pine. We used electroporation successfully in the evaluation of promoter and gene constructs. Putative transformants have also been recovered following *Agrobacterium*-mediated transformation of somatic embryo material and from *Agrobacterium* co-cultivations with zygotic embryo cultures capable of being regenerated into complete plants.

Agrobacterium-mediated transformation was also developed to a practicable level, with transgenic zygotic and somatic embryos of white spruce produced.

3.3.4 Technology Contacts and Collaborations

Among our important external contacts were:

* University of Calgary (including NSERC placement) embryogenesis; transformation.
* University of Saskatchewan (including shared NSERC): embryogenesis.
* University of Alberta: gene expression measurement.
* Forestry Canada/Victoria: micropropagation of pines.
* Stange Canada Ltd.: guest research.
* Weyerhaeuser Canada Ltd.: micropropagation of spruce.

All project members participated strongly in the 1988 conference of the International Conifer Tissue Culture Working Group, held in Saskatoon, and the group maintained a large number of links and collaborations through the year.

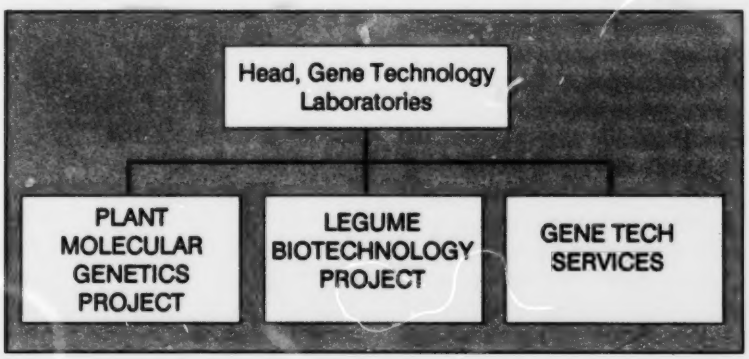
4. Gene Technology Laboratory

Laboratory Head: Dr. W. L. Crosby

4.1 Overview

The Gene Technology Laboratory of the Institute was established only in 1985, and since then has undergone a remarkable growth to its present complement of nearly fifty staff and guests. The section is concerned mostly with recombinant DNA technologies applicable in plant genetic engineering. During 1988/89 the original five-year plan for development was completed and an array of staff expertise, equipment and DNA services was fully operational and being used by many research organizations. Completion of an integrated DNA synthesis/sequencing laboratory brought the number of working units to twelve. Growing intramural and extramural collaborations contributed to the strength of the program this year.

The **plant molecular genetics** project continued to be the main activity of the Laboratory. In summer 1988 the plant productivity genes activities were put into a wind-up phase and a new project in **legume biotechnology** was begun. The general organizational framework of the Laboratory is shown in the figure below.



4.2 Plant Molecular Genetics Project

4.2.1 Project Personnel

Research Officers: W. L. Crosby (Project Leader), S.M. Hemmingsen, L.E. Pelcher, G. Selvaraj, J.L. Taylor

Research Associates: A.G. Good, A. Hussain, L. Klimczak, G. Penner, D.S.S. Raju, L. Cloney

Guest Researchers: M. Balasubramanian, L. Boyd, R. Jain, K. Rozwadowski, A. Woytowich

Technical Officers: C. Barber, C. Bennett, I. Borgmann, J. A. Condie, J. K. Hammerlindl, R. Hirji, D. Kneeshaw, M. Olson*, D. Schwab, M. Wood*

Summer Students (1988): D. Neustaedter, A. Nikiforuk

*Term appointee

4.2.2 Introduction

During 1988/89 we continued our objective of exploring new and exploitable technologies governing plant gene regulation, our aim being to develop strategies for the directed modification of crops. *Brassica* species remained our target system.

We dealt with four interrelated topics:

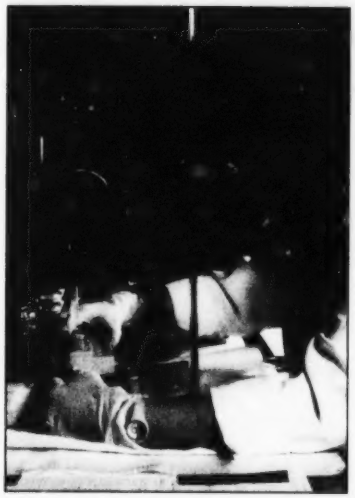
*Protection from environment stresses (herbicides, water/salt stress);

*Protection from other organisms (insects, fungi)

*Plant development genes

*Expression vector technology

The year saw establishment of two new laboratories, for fungal protection research and for DNA synthesis and sequencing.



Technical officer Colleen Bennett works behind a protective shield while labelling a DNA fragment with radioactive P32.

4.2.3 Research Progress

4.2.3.1. Plant Protection From Environmental Stresses

Transgenic Herbicide Resistance

Point mutations in the gene for acetolactate synthase (ALS) result in resistance to sulfonylurea herbicides. Herbicide resistance in canola is a desirable agronomic trait for weed control. Our objective is to create transgenic canola plants with herbicide resistance, based on ALS techniques; for 1988/89 the goal was to describe the genes for this.

We reported 1987/88 the isolation of a genomic ALS clone from *B. napus* which was 83% similar to that of *Arabidopsis* at the deduced amino acid level. With a published report of the specific mutational lesion, and a perfect conservation of primary structure in the region of this mutation, we used a synthetic oligonucleotide to derive the site-directed mutant derivative of PBI-201 which mimicked the published mutation in *A. thaliana*. The *B. napus* mutant was subsequently shown to confer tolerance to chlorsulfuron in transgenic tobacco, thus confirming its identity as an expressed ALS gene.

This year we isolated a full-length ALS cDNA from *Brassica napus*. This 2215 base-pair intron-less sequence contains a 1911 base-pair open reading frame 93% similar to a genomic clone previously isolated by this project. The cDNA defines an ALS gene family for *B. napus* where at least two members are structurally distinct, and as the first ALS cDNA ever described provides the first **direct** evidence for lack of introns in higher eukaryotic ALS genes.

Examination of the upstream regulatory regions from our ALS constructs from *A. thaliana* and *B. napus* revealed puzzling patterns of gene activation in both transient and stable transgenic experiments, suggesting complex promoter structure. An enhancer-like sequence was identified and found to be precisely conserved between *B. napus* and *A. thaliana*.

During 1988/89 we succeeded in observing herbicide resistance in transformed tobacco plants and in transformed *B. napus* plants; however new high-activity promoter constructs have not yet been applied in complete plant tests.

Salt and Water Stress Responses

Western Canadian crops frequently face drought and salt stresses, responding by inducing specific gene activities which presumably help the plant cope biochemically. We have studied salt-induced stress in the notably salt-tolerant plant *Kochia scoparia*. The objective is to identify specific genes involved in the plant response.

During 1988/89 we identified from the bacterium *Escherichia coli* two genes that confer salt-tolerance: choline dehydrogenase and betaine aldehyde dehydrogenase. Physical and genetic mapping, and sequencing, have all been completed.

In a parallel approach we began to compare protein profiles of salt-induced and control *K. scoparia*. This promising work is continuing.

Experiments on water stress response in barley (*Hordeum vulgare*) were largely completed in 1988/89, with some of the work being published. A new participant in the plant response was discovered, alanine aminotransferase, and work to fully describe this system was underway at year-end.

4.2.3.2 Plant Protection From Other Organisms

We chose to focus on insect and fungal protection because these are most important in the oilseed *Brassicas*. The existence of specific known genes which can give rise to "resistant" plant phenotypes is the starting point, our objective being to create transgenic plants with useful levels of resistance.

Fungal experiments are now in their third year, and for the first time a sizeable team has been assembled on this problem. The team has pursued purification of plant chitinases -- known as anti-fungal agents -- and isolation of genes that encode them. We purified a *B. napus* chitinase and raised polyclonal antibodies in preparation for immunoscreening of cDNA libraries. *Arabidopsis thaliana* was also found to have chitinase activity.

During 1988/89 our fungal work extended to examining protection against *Leptosphaeric maculans* ,the fungus causing blackleg disease in *Brassica*. First vector constructs were completed by year-end.

Insect experiments continued to focus on special NRC-proprietary forms of genes for *Bacillus thuringiensis*-type toxins, originating at the Biotechnology Research Institute in Montreal. Modifications of this gene were made for plant use, and substantial advances were made in the promoter methods for high expression of these genes in *Brassica*. Most of our effort during 1988/89 was directed at structure/function experiments with modified gene forms and we identified a series toxic to Bertha armyworm .

4.2.3.3 Plant Development Genes

Plants, like other lifeforms, develop by cell division and differentiation. These processes are gene-controlled and therefore susceptible to biotechnological interventions. Our main objec-

tive is to identify one or more plant genes functionally homologous to cell-cycle genes already known from yeasts.

We succeeded in introducing the gene WEE1$^+$ from the yeast *Saccharomyces pombe* into tobacco and recovered transgenic plants containing the new gene. No visible phenotype could be associated with the gene. It is possible that an observable phenotype can exist but that the level of expression of WEE1$^+$ in our tobacco was too low to produce it. We are now pursuing over-expression to test this idea.

Follow-up to earlier work on chaperonin mediation of gene products took substantial time during 1988/89. Through complete cDNA sequencing we found that *Brassica napus* chaperonin is composed of two quite dissimilar units. Isolation and sequencing of partial cDNA sequences encoding chaperonin units in *A. thaliana* were also carried out. Unusually severe problems of instability of materials have hindered progress on structure/function studies of chaperonin units.

4.2.3.4 Expression Technology for Transgenic Plants

The importance of gene expression in transgenic systems made this an important area of study again in 1988/89. The objective is to create and make available improved constructs for transfer and regulation of genes.

A series of novel bifunctional fusion genes was isolated for several selectable and assayable functions including beta-glucuronidase (GUS) and neomycin phosphotransferase (NPT) activities. The bifunctionality of the fusion peptides from these genes was rigorously demonstrated. We used them successfully both in bacteria and in higher plants. The series is being extended, and patent protection is being sought.

Duplicating the enhancer sequences of the 35S promoter of cauliflower mosaic virus increased the expression of this promoter through enhanced transcription, and it is now possible to generate high-expression plant promoters by similar means. During 1988/89 we incorporated the apple mosaic virus RNA4 untranslated leader sequence into plant promoters and assessed the resultant promoters in tobacco, conifer and monocot cell systems. We observed "order-of-magnitude" increases in marker gene expression in all these systems. The work is currently being extended to transgenic whole plants.

4.2.4 Technology Contracts and Development

The project engaged in many research collaborations during the year, with other Canadian and international groups:

*University of Warwick (U.K.) and University of Arizona: molecular chaperones.

*University of Calgary: ALS-based herbicide resistance.

*University of Guelph: *Brassica* repetitive DNA elements.

*University of Saskatchewan: Genetics and metabolism in *Arabidopsis*.

*Queen's University: Expression of yeast genes in plants.

*Biotechnica Canada Inc.: Plant lipase genes.

*Agriculture Canada/Saskatoon: Blackleg resistance.

Dr. Janet Taylor was recipient of a 1989 President's Fund award for basic research, the fourth such award to project members in three years. Project scientists gave six keynote talks at scientific meetings along with more than fifty other lectures and presentations.

Construction of a new DNA synthesis/sequencing laboratory, and acquisition of an ABI model 370A automated sequencer, have substantially improved the technical base of the Institute. These services were also made available at cost to numerous extramural users.

4.3 Legume Biotechnology Project:

4.3.1 Project Personnel:

Research Officers: J.D. Mahon (leader), S.L.A. Hobbs, L.M. Nelson

Technical Officers: D. Baliski, C. DeLong, M. Anderson, C. Knapp, M. Swartz (temporary)

Research Associates: C. Chanway (to 24 June 1988), M-F. LeGal (to 13 September 1988), Jennifer Jackson (from 25 August 1988)

Guest Researchers: S. Sprout, F. Joseph, G. Sailaja

Summer Assistants: K. Barrett, H. Bourassa

4.3.2 Introduction

The overall objective of the Legume Biotechnology Project is to improve crop legume performance through the application of biotechnology. The objectives for 1988/89 were: (i) to develop tissue culture and gene transfer technologies for grain legume crop species; and (ii) to complete other ongoing research to a state suitable for publication or transfer.

The establishment of this new project in June 1988 recognized both our group's continuing committment to the agricultural improvement of grain legume crops, which account for annual farm revenues of over $600 million in Canada, as well as the need for research on directed genetic modification of these species. Thus during the year, the group committed an increasing proportion of its resources to research aimed at the development of transgenic technology for grain legumes, and concluded work in earlier research areas.

Virtually all grain legumes have proven to be recalcitrant with respect to the production of transgenic plants by any of the known methods. Nevertheless, because pea is known to be susceptible to infection by *Agrobacterium*, we initiated transformation work using *Agrobacterium*-based vectors to transfer genetic information to pea. For success, the following are required:

- protocols for the regeneration of plants from a cell or small number of cells.

- a demonstration that the above cells are susceptible to *Agrobacterium* infection and that T-DNA is integrated into their genomes.

- the successful integration and expression of marker genes in transformed plant tissues

- the demonstration of stably inherited expression of genes in regenerated plants

4.3.3 Research Progress in 1988/89

4.3.3.1 Regeneration and transformation of pea cells

Regeneration of complete plants from cells which are accessible and susceptible to *Agrobacterium* is necessary for this method of plant transformation. Although regeneration from transformed callus tissue is possible in many plant species, pea callus rarely generates shoots. Therefore several methods of direct regeneration from pea explants, including the previously reported methods for regeneration from immature leaflets and apical meristems were assessed for their potential use in transformation.

Regeneration from immature leaflets was improved by finding appropriate genotypes of pea and optimal experimental conditions. Leaflet age proved crucial, with explants from the third leaf of 2 days-old seedlings or the fourth leaf of 4 days-old seedlings best producing shoots. Shoot apices could also be induced to form large numbers of shoots. Tissues derived from both of these kinds of explant can now be maintained in conditions which allow continuous production of shoots over a long period of time.

Explants from other parts of the plants including epicotyls, hypocotyls, roots and cotyledons were examined without evidence of shoot production.

Pea transformation - All 35 pea genotypes which we examined showed *Agrobacterium* gall formation. The number and size of tumors on explants were dependent on both the genotype and the strain.

Fifteen strains of wild *Agrobacterium tumefaciens* (from Agriculture Canada) varied in their abilities to grow on recommended media. The best general-purpose media, on the basis of lag time and yield, were a nutrient broth yeast extract sucrose medium and YEB (yeast extract-beef extract) medium. These strains were tested for tumor production on immature leaflets of selected pea genotypes.

The transmission and expression of T-DNA in effective combinations was confirmed by the growth of axenic cultures of tumor tissues from the wound sites on hormone-free medium and by production of the opines specific to the *A. tumefaciens* strain used. Integration of the T-DNA into the pea genomes was verified by Southern blot analysis of callus DNA, showing conclusively that *Agrobacterium* infects pea tissues and effects the transfer of T-DNA into the pea genomes with expression of the bacterial genes. Thus there is no inherent barrier to the use of *Agrobacterium*-mediated transformation to introduce foreign genes into peas.

Disarmed *A. tumefaciens* strains with marker-containing binary vectors were cocultivated with pea explants under various conditions, and by year-end, green and healthy regenerated shoots were growing on the kanamycin selection medium. Experiments aimed at obtaining transformed pea callus have not yet produced enough material for assays to detect successful transformation.

4.3.3.2 Plant Growth Promoting Rhizobacteria (PGPR)

Soil bacteria which enhance plant growth by producing plant growth hormones, controlling nutrient availability or reducing deleterious microorganisms have been known for many years and at least 3 commercial inoculants are already available with demonstrated utility. Other examples are known to be in the process of registration and there appears to be a renewed interest in the area. During 1988/89 our involvement in this research topic was winding down rapidly.

Wheat growth promotion: *Bacillus* isolates previously shown to produce a variety-specific stimulation of wheat root growth in sterile test tube assays were further examined in sterile Leonard jars and non-sterile pots with similar positive results. The stimulation was not dependent on the bacterial medium and inoculation with 10^7 cells/mL was optimal. These isolates were tested with Katepwa and Neepawa wheats in a small field plot. One inoculant increased shoot growth by 52% while the other gave no growth increase. The role of bacterially - produced indoleacetic acid in the growth effect was not clarified.

Lentil growth promotion: Bacterial isolates from the roots of three cultivars grown in a local field which had been in continuous lentil production for at least four years were screened for their effects on the growth of lentils. Twenty-one isolates were screened on the cultivars from which they had been isolated and three were selected as positive. One of these was identified as *Serratia plymuthica*.

4.3.3.3 Genetic Variability in Pea Photosynthesis

In 1988/89 we concluded this work.

Our research on genetic control of photosynthesis had earlier established that genetic variability among pea genotypes is reproducible and highly heritable; that this variability is related to plant growth in field stands; that much of this variability in photosynthesis is due to high levels of RUBISCO protein in the leaves; and that the RUBISCO levels are highly correlated with the abundance of mRNA for its small subunit during development of the photosynthetic apparatus.

Pea genotypes having different amounts of the RUBISCO protein showed correlated differences in the amount or RUBISCO small sub-unit mRNA during greening. This suggested that genotypic as well as environmental effects on RUBISCO were caused by differences in transcription which could reflect either different gene doses, coordinate alterations in transcription of all members of the RUBISCO small sub-unit gene family, or variations in the transcription from individual genes.

Digestion of the genomic DNA of twelve pea genotypes using EcoRV (which has a single cutting site in each of the pea genes for which sequences have been reported) gave five fragments from all genotypes but one, which had only four. This pattern did not correlate with the overall level of transcripts; differences in gene dosage are therefore not the cause of transcript variability.

All of the rbcS mRNAs are virtually identical in their coding regions and only slightly different in 3' and 5' non-translated sequences. Therefore quantitation of mRNA from specific genes was done using S1 nuclease analysis. However, some transcripts were relatively discontinuous, with some genotypes showing scant expression and others a considerable amount.

Since reproducible variability in pea photosynthesis was discovered at PBI, we have maintained activity in backcrossing high and low photosynthesis plants with the commercial pea varieties Homesteader and Trapper. In field tests, the selected progeny from the high photosynthesis donor parents were superior in photosynthesis to those from low donor parents. This demonstration that genetic differences in photosynthesis can be maintained after several cycles of backcrossing in plants which are similar in other traits, provides evidence that genotypic differences in photosynthesis are due to variations in genes directly involved in the control of photosynthesis.

Dr. Shaun Hobbs estimates fragment sizes of DNA.

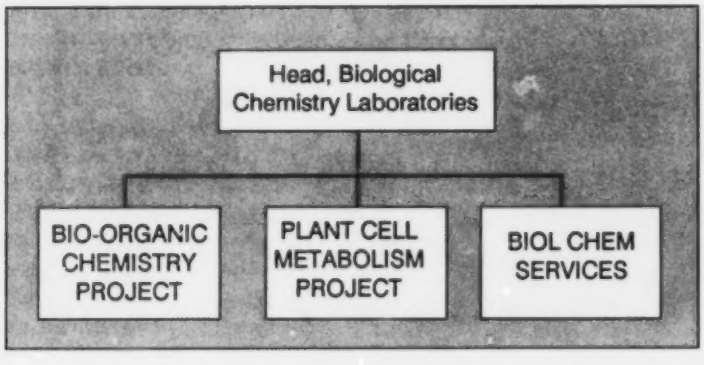
5. Biological Chemistry Laboratory

Laboratory Head: Dr. E. W. Underhill

5.1 Overview

PBI's Biological Chemistry Laboratories provide a strong "molecular" element for the Institute's molecular biology activities. The ten units have excellent resources for bio-organic synthesis and analysis and for protein and other biochemical manipulations. The Institute's long tradition of excellence in phytochemistry and plant biochemistry was continued in 1988/89 through the two projects of the Laboratory. The **bio-organic chemistry** project pursued two main activities : research on abscisic acid analogs and research on the biotechnical modification of lipids in oilseed plants. The **plant cell metabolism** project continued to produce technology for the industrial use of plant cell cultures as sources of phytochemicals.

Thanks to the great numbers of guest researchers hosted in 1988/89, PBI's biological chemistry workforce surpassed fifty for the first time. Both of the Institute's industrial "incubator" laboratories are located on this Laboratory's premises. The general organization of the Laboratory is illustrated schematically below.



5.2 Bio-organic Chemistry Project

5.2.1 Project Personnel

Research Officers: E. W. Underhill (Project Leader), S.R. Abrams , J.W.D. GrootWassink , N. Lamb - (Term, from June 88), S.L. MacKenzie, M. Mazurek, G.P. Slater , N. Weber (VRO to 31 October 88).

Technical Officers: M. E. Giblin, L. R. Hogge, L. L. Hoffman, A. D. Kolenovsky, D. J. Olson, D. W. Reed, A. C. Shaw, D. M. Tenaschuk

Research Associates: J.C. Jain , R. Martel , M. S. Pedras, K.A. Pivnick , D.C. Taylor, L.W. Thomson, R. J. Weselake

Guest Researchers: G. D. Abrams, V. K. Bansal, C. Craig, L.Davin, L .Gusta, G. Haughn, B. J. Jarvis, L. A. Nelson, R. Power, M. Reaney .

Summer Students 1988: M. Chester, B. Pittendrigh, R. Power

5.2.2 Introduction

The project's research program for 1988/89 encompassed studies on:

(1) the chemistry and biological activity of compounds involved in plant stress and development,

(2) purification and characterization of plant lipases, and,

(3) alteration of heritable plant traits not attainable by plant breeding.

The output and accomplishments of the project during the year were again very high with 33 papers published in refereed scientific journals. In addition, members of the team presented four seminars and 15 posters and papers at scientific meetings. New joint ventures and collaborations were initiated during the year between the project's research officers and university, industry and government laboratories. The project's mass spectrometer facilities were upgraded during the year and all of the Institute's research groups made use of the new instrument.

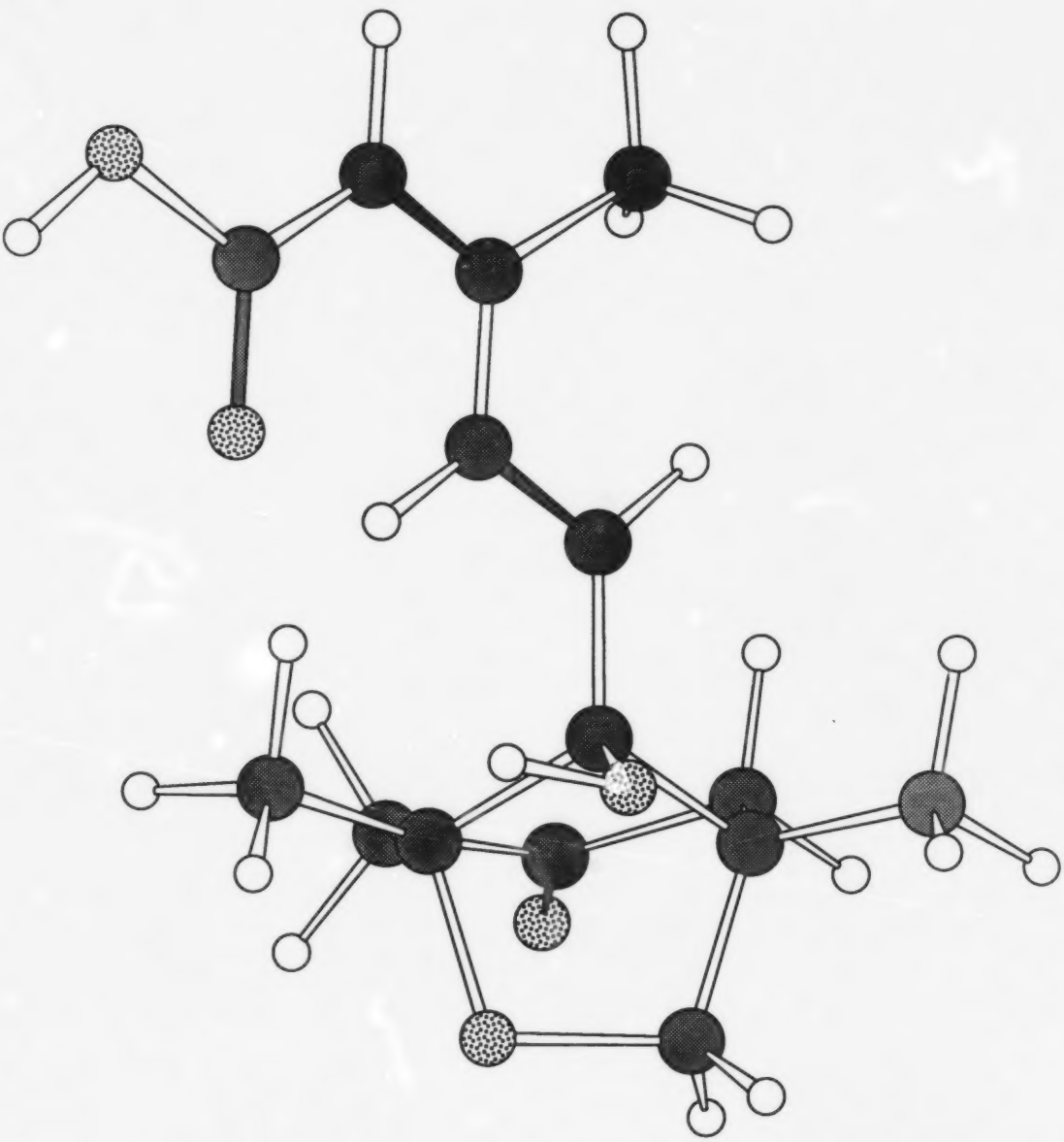
5.2.3 Research Progress

5.2.3.1 Chemistry and Biological Activity of Abscisic Acid Metabolites and Analogs

The specific objective of this research is to control growth and development of plants by manipulating processes regulated by the hormone abscisic acid (ABA) or its analogs.

The plant hormone abscisic acid is involved in the regulation of many phases of plant growth and development. Analogs of the hormone were synthesized by systematic variations of the functional groups of the ABA molecule. Biological testing was carried out by numerous collaborators with expertise in dormancy, seed development, plant protection from drought and cold, and in plants ranging from cereals to conifer species. ABA-related chemicals have been found to have a range of ABA and anti-ABA activities. Compound PBI-16 proved more potent than ABA in inhibiting seed germination. Compound PBI-11 promoted germination, and other analogs promoted root growth, but not shoot growth. Investigations into practical uses in agriculture and forestry expanded through testing agreements with collaborating groups, and patents were filed to protect the newly-investigated analogs.

Understanding the mechanism of action of ABA is important in its own right, and relates directly to the development of plant growth regulators. The biological activity of precursors and metabolites of ABA was examined using plant cell cultures to study the metabolism of ABA and its analogs. The first practical total synthesis of the major metabolic product, phaseic acid, has been accomplished. Biological testing of germination and cold hardiness studies is underway in several Canadian laboratories.



Structure of a phaseic acid biologically active metabolite of abscisic acid. The first total synthesis of phaseic acid has been accomplished.

5.2.3.2 Protection of Crops From Insect Pests

The immediate objective is to identify compounds produced by canola, mustard, stinkweed and their role in chemical communication with the diamondback moth. Longer term goals include extension of research to other crucifer pests and transfer of results to molecular biologists/plant breeders for development of pest-resistant crops.

Combined gas chromatography-mass spectrometry analysis indicated significant differences and similarities in volatiles from homogenates of brown mustard and stinkweed which may explain why the latter is not a host plant of the diamondback moth and the former is. Use of preparative gas chromatography and monitoring of fractions by electroantennogram analysis was effective in indicating bio-activity in fractions and individual components. Plant extracts (of intact plants and homogenates) which induced a response on the electroantennogram analysis also were attractive in Y-tube bioassays and attracted diamondback moths in field tests. Similar results were obtained with individual components of plant volatiles extracts. Synergistic effects resulting from combination of various extracts and fractions indicate that "green leaf" and glucosinolate derived volatiles are involved in attraction of the diamondback moth to brown mustard plants.

5.2.3.3 Plant Lipid Biotechnology

1. Lipases

Acylglycerol acylhydrolases (lipases) have long been important in several industrial sectors, specifically the chemical, food, and pharmaceutical industries. Currently, microbial lipases are exclusively used in these applications. However, there is a need for lipases having a well defined specificity, and improved thermal and organic solvent stability. Plants have been a conjectural source of such enzymes.

The objectives of this research were to isolate and characterize canola *(Brassica napus)* lipases and to generate anti-lipase polyclonal antibodies which could be used to isolate the encoding *B. napus* genes.

B. napus liposomal and microsomal lipases were partially purified and characterized and an antibody to the liposomal lipase was made available for screening of cDNA expression libraries. A 'soluble' lipase has been purified more than 1000-fold from the microsomal supernatant. Ether lipids have been determined to be suitable substrates for the study of lipase positional specificity.

Seed Storage Triacylglycerol Assembly

High erucic acid rapeseed has a theoretical breeding limit of about 66% erucic acid in its seed oil, since erucic acid is virtually excluded from the middle (sn-2) position on the glycerol backbone of triacylglycerols. Seed oils rich in trierucin would be desirable as sources of specialized lubricants and useful in the industrial production of plastics, nylons and detergents. Erucic acid is classed as a "strategic material".

The long range goal of this research is the development, through recombinant DNA technologies, of new plant varieties capable of producing specifically designed seed oils not attainable using conventional breeding methods. The immediate goals for 1988/89 were elucidation of the biosynthetic pathway through which erucic acid is incorporated into seed storage lipids and the isolation and purification of pathway enzymes.

Microspore-derived embryos of *B. napus* cv Reston have been developed as a novel experimental system for the study of triacylglycerol biosynthesis. To date the system has demonstrated a very high capacity to incorporate erucyl moieties into triacylglycerols; supplied with 1,2-dierucin, homogenates of the embryos produced trierucin. The embryo system provided opportunities to explore the complexities of triacylglycerol biosynthesis and to provide a model for purifying and characterizing the enzymes necessary for eventual construction of chimeric genes for plant transformation.

This line of research seems very promising for future agro-industrial development in

Canada and is slated for expansion in the years ahead.

5.2.3.4 Elimination of Glucosinolates

An important application of molecular biology in agriculture is the development of new chemical phenotypes of economically important crop lines through transgenic expression of foreign genes. However, the technology to genetically engineer higher plant phenotypes devoid of specific components that restrict the usefulness of a particular plant product awaits development; it has many applications in agriculture. The system adopted for this study was the elimination of glucosinolates from *Brassica* species.

The long range goal of this research is the development of methods to block the expression of endogenous genes in plants. The specific objectives for 1988-89 included (1) development of a chromogenic assay for glucosinolate formation in plants, (2) purification of enzymes involved in glucosinolate formation, and (3) assays of *Arabidopsis thaliana* for mutational defects in glucosinolate formation.

A chromogenic marker was developed to assay glucosinolate biosynthesis in whole plants and *in vitro* cultures. We established that exogenous aldoximes not only are converted to their corresponding glucosinolates in crucifers but also to sulfatoglucoside conjugates in a much wider range of plant species. The glucosyltransferase and sulfotransferase enzymes occur in the cytoplasm of suspension-cultured cells. We achieved purification of the last enzyme of the glucosinolate pathway, desulfoglucosinolate sulfotransferase, by improving the stability in buffer solutions, and by application of affinity chromatography. A protocol was developed which permitted purification to homogeneity of the penultimate enzyme in the glucosinolate pathway, a thiohydroximate glucosyltransferase. This enzyme was characterized as to molecular weight, inhibitors and cofactors.

5.2.3.5 Biological Chemistry Services

The Institute's main nuclear magnetic resonance (NMR) instrument, a Bruker AM-360 MHz (1H and 13C probes) used in routine analysis for structural characterization and/or structural elucidation of synthetic and natural compounds, acquired a 13C-1H dual probe. This probe allowed more rapid determination of spectra, and use of long-range 1H-13C couplings for structural elucidation.

During 1988/89 our new mass spectrometer (VG 70-250 SEQ Hybrid MS/MS system) was reinstalled in the renovated Mass Spectrometry Laboratory. The other two mass spectrometers (Finnigan 4500 and Finnigan 3300 systems) were also moved to this location. This new laboratory centralizes the mass spectrometry facility within PBI and has addressed problems of safety, noise and ventilation. This instrumentation is capable of analyses using electron impact, chemical ionization, fast ion bombardment or thermospray ionization. Over 1350 analyses were performed in 1988/89, many for extramural users of the facility.

5.3 Plant Cell Metabolism Project

5.3.1 Project Personnel:

Research Officers: W.G.W. Kurz (leader), J.J. Balsevich, V. DeLuca, R.T. Tyler

Technical Officers: G. Bishop, K.B. Chatson, D. Dymond, J. Magus, B.D. Panchuk

Research Associates: S. Chevadej, N. Palva, I. Roewer

Guest Researchers: D. Epp, J.A. Fernandez, L. Friesen, K. Giles, C. Hill, D. Novakovski, J. Park, R. Power, D. Songstad, S. Tallevi, L. Toivonen

Summer Assistants: M. Dethier, M. Levy

5.3.2 Introduction

The objectives of the project are to investigate metabolic pathways in plant cells and to develop methods for their control and for the commercial production of phytochemicals by *in vitro* culture processes.

5.3.3 Research Progress in 1988/89:

During the year the Project generated eight scientific publications and one patent. Patents and"know-how" created in previous years were licensed to two biotech companies [Vipont Canada Ltd. and Intrinsic Research & Development Inc.] and members of the Project are actively involved in aiding these two companies as well as a third one, Bio-Mega in the development of biotechnical processes. The group's involvement in NRC's interdivisional effort to evaluate surface immobilized plant cells has continued.

5.3.3.1 Elicitation of Secondary Metabolism:

Objective: to suppress elicitation variation in sanguinarine production with poppy.

Soluble elicitor preparations (hydrolsates of fungal mycelium cell wall) allowed us to eliminate variability in elicitor dosage and to determine that much of the variability in sanguinarine production by elicited poppy cell cultures lies with the cultures. Inconsistent culture aeration in shake flasks proved to be largely responsible for this variability. This effect should not pose a significant problem in larger-scale systems, however chitin (poly-N-acetylglucosamine) and its hydrolysates were effective elicitors of sanguinarine production in poppy cultures; N-acetylglucosamine (1-mer), chitobiose (2-mer) and chitotriose (3-mer) showed no elicitor activity.

We tried to determine the mechanisms of sanguinarine release from elicited cells. Active transport, leakage due to temporary loss of membrane integrity, and diffusion from dead cells all contribute to release of sanguinarine. We were unable to measure reliably the relative contributions of these mechanisms.

5.3.3.2 Enzymology of Alkaloid Synthesis:

We examined the expression of two genes which catalyse steps in vindoline biosynthesis. We hope to insert these genes into undifferentiated plant cells and thus induce synthesis of important metabolites normally restricted to the differentiated state.

Tryptophan decarboxylase (TDC) from *Catharanthus roseus* was purified to homogeneity and antibodies of high titer were prepared from poly (A) + RNA isolated from developing seedlings. We established the presence of TDC mRNA. The cDNA library was screened with monospecific anti-TDC antibodies. One clone so obtained was sequenced in both orientations; The deduced amino acid sequence for the gene product shared 190 units out of a possible 494 matches with L-aromatic amino acid oxidase decarboxylase from the fruit fly *Drosophila melanogaster* - 40% homology. The expression of cloned *C. roseus* TDC-ß galactosidase fusion protein in *E. coli* gave an enzyme capable of

decarboxylating tryptophan, evidence that indeed the cloned sequence encodes authentic TDC.

Acetyl Co-A-deacetylvindoline O-acetyltransferase from *Catharanthus roseus* was similarly examined but we found no homology with the putative peptide.

5.3.3.3 Alkaloid biosynthesis:

The objective was to demonstrate the pathway of vindoline biosynthesis to increase the production of this substance.

We established hairy root cultures of *Catharanthus roseus* by infection with six different Agrobacterium rhizogenes strains. Two plant varieties responded differently to infection. Plants cloned from normal flowerless varieties were screened for indole alkaloid production. The alkaloid spectra were qualitatively but not quantitatively very similar to those of normal plant roots. No vindoline or deacetyltransferase activity could be detected in any of the cultures studied. Next, two root clones were examined for their growth and alkaloid accumulation during a 26-day culture period. Alkaloid accumulation paralleled growth in both clones.

We established and analysed cell suspensions of a high-alkaloid flowerless mutant line of *Catharanthus roseus* . Overall alkaloid yields were typical but no catharanthine was produced in the suspension cultures. This paralleled results with entire mutant plants which in contrast to normal plants do not accumulate catharanthine in roots, but only in leaves.

The distribution of alkaloids in *C. roseus* plants was of interest for possible field production. Youngest leaves contained the highest concentrations of catharanthine and the lowest concentrations of 3', 4'- anhydrovinblastine. More mature leaves contained higher concentrations of 3', 4'-anhydrovinblastine and reduced concentrations of catharanthine, but vindoline concentrations remained constant. Our flowerless variety exhibited very dynamic alkaloid accumulation patterns ranging from extremely high vindoline levels in the younger leaves to virtual absence of vindoline.

Dr. Bob Tyler checks periwinkle (Catharanthus roseus) blossoms in the PBI greenhouse.

5.3.3.4 Evaluation of surface immobilization of plant cells

Objective: To produce secondary metabolites using new immobilized plant cell systems. This is an interdivisional comprehensive program with the Biotechnology Research Institute.

Two model systems, *Catharanthus roseus* (indole alkaloids without elicitation) and *Papaver somniferum* (benzophenanthridine alkaloids upon elicitation) were compared as suspension and immobilized cultures. Even gentle stirring-in particularly after elicitation had a damaging effect, and in suspension cultures a great number of cells fixed themselves to reactor walls and fixtures. Therefore immobilization was the preferred mode of cultivation. It also performed better for medium exchange, and for removal of excreted products and excess elicitor. For both species immobilized cultures regularly outperformed corresponding suspension cultures.

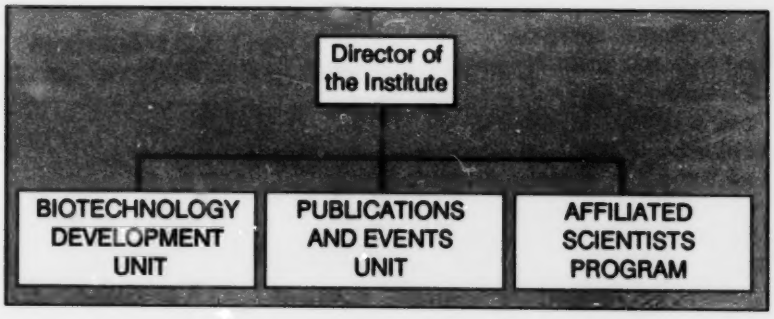
6. Business and Outreach Activities

6.1 Overview

The Plant Biotechnology Institute carries out networking and communications activities in keeping with the National Research Council's program of scientific information and assistance. Our business program gears our facilities and expertise to the development needs of the nation and puts new technologies into action. Through outreach activities we ensure that our capabilities are known to Canada's research communities and accessible by members of the plant biotechnology community.

"Doing business" has become an important part of research at PBI, so that there are no longer any laboratory groups without important extramural collaborations underway. The siting of industrial research projects at the Institute reached a new high in 1988/89, recognizing the unique capabilities which exist here. Moreover, this trend will probably continue to accelerate in 1989/90.

Business & Outreach



6.2 Business Activities

6.2.1 Biotechnology Development Unit

This unit, headed by Dr. W. G .W .Kurz, includes a spectrum of activities all having a science/business interface. These activities include industrial liaison (Dr. Louise Nelson), international liaison (Dr. Kurz), contracting-out through the Plant Biotechnology Fund (Dr. Kurz and J. Sylvan Katz), and development of agreement terms for technology transfer and licensing. The unit also has responsibilities for liaison with extramural funding sources.

The Plant Biotechnology Fund budget declined in 1988/89 because of changing cost-benefit ratios and in view of continuing fiscal difficulties at NRC. Contracts for $457 894 worth of research constituting parts of the Institute's core project activities were completed in 1988, and a further $260 000 in contracting was approved for 1989. Contracts are awarded competitively: following issue of specifications and cost limits by the Fund committee on behalf of the project groups, offers of performance from prospective contractors are received through Supply and Services Canada and evaluated in committee. Contracts are for one year.

Contracts performed for PBI via the Plant Biotechnology Fund during 1988/89 were:

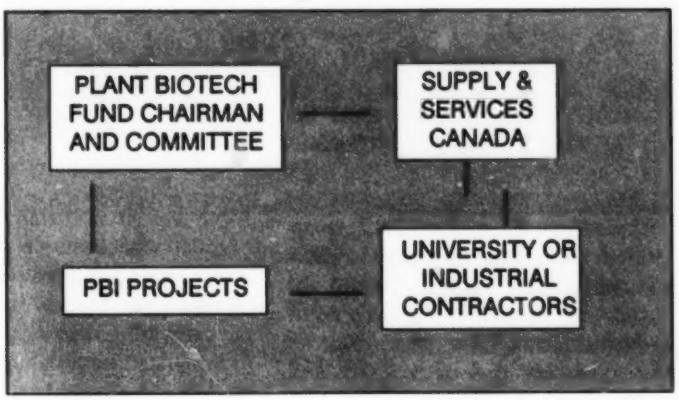
Characterization and heterologous expression of genes encoding herbicide resistance in Brassica napus (phase 2).
Contract value : $120 000
Principal investigator : Prof. Maurice M. Moloney, University of Calgary
PBI Scientific Advisor : Dr. W. L. Crosby

Abscisic acid analogs.
Contract value : $110 000
Principal investigator: Dr. Lawrence V. Gusta, Crop Development Centre (U of Sask)
PBI Scientific Advisor : Dr. S. R. Abrams

Extrachromosomal elements of Brassica species - Phase II
Contract value: $67 000
Principal investigator: Dr. H. Bertrand, Dept. of Microbiology, University of Guelph, Guelph, Ontario
PBI Scientific Advisor: Dr. G. Selvaraj

Biochemical genetics of glucosinolates in Arabidopsis thaliana and Bassica spp.
Contract value: $100 940
Principal investigator: Dr. G. Haughn, Dept. of Biology, University of Saskatchewan, Saskatoon, Sask.

Contracting out PBI research


PLANT BIOTECH FUND CHAIRMAN AND COMMITTEE
SUPPLY & SERVICES CANADA
UNIVERSITY OR INDUSTRIAL CONTRACTORS
PBI PROJECTS

Preparation and supply of wheat sperm cells
Contract value: $60 000
Principal Investigator: Dr. D. Cass, Dept. of Botany, University of Calgary, Calgary, Alberta
PBI Scientific Advisor: Dr. A. J. Cutler and Dr. M. Saleem

The Unit negotiates licenses for the exploitation of protected technologies from PBI. At March 1989 the Institute held seventeen patents in force, had filed applications for two more, and had six other inventions registered with agents of the National Research Council with a view to filing patents on them. Patents granted or filed for during 1988/89 were:

- J. Archambault, B. Volesky and W.G.W. Kurz: ***Surface immobilization of plant cells.***
USA Application #186 635 (27 April 1988)
Australia Application #15054/88 (27 April 1988)
Europe Application #88.304 077.6 (5 May 1988)
Canada Application #537 315 (15 May 1988)
Japan Application #116 794/88 (13 May 1988)

- S. R. Abrams and L. Gusta: ***Abscisic acid related plant growth regulators and germination promoters.***
USA Application #280 102 (1 December 1988)

- E. W. Underhill, J. W. Wong, P. Palaniswamy, W. F. Steck and M. D. Chisholm: ***Attractants for Fall Cankerworm moths.***
Canadian Patent #1 231 983 (1988)

- V. DeLuca, N. Brisson and W.G.W. Kurz: ***A tryptamine-producing tryptophan decarboxylase gene of plant origin.***
USA Application #314 879 (24 February 1989).

6.2.2 Joint Research under Agreements

Joint research by contracting-in and shared-cost ventures increased this year. Through a continued Agreement with the Saskatchewan Crop Development Centre, the Institute again had access to agronomic test facilities and expertise for follow-up work to in-house production of prototype plants.

Agreements with Philom Bios Inc. and Vipont Laboratories Canada Inc. (both headquartered at Saskatoon) made the Institute a home for research groups of these growing companies. The interest areas are growth promoting organisms and the biotechnical production of certain alkaloids with therapeutic value. At year-end these partners of PBI had a total of 15 research employees on-site.

Work on plant lipases and on triglyceride assembly genes was continued jointly with the Biotechnology Research Institute (Montreal, Que) and Biotechnica Canada Inc.(Calgary, Alta), assisted by the NRC Biotechnology Contributions program. The goal of this research is to develop recombinant DNA technology for modification of oilseed lipid profiles. In a second contributions-aided project, BRI and PBI began work with Bio-Méga Inc. (Montreal, Que) on production of gingkolide derivatives from plant cell systems.

PBI undertook two IRAP-supported joint research projects. A venture with the Balco-Canfor Reforestation Centre (Kamloops, B.C.) was set up for application of abscisic acid analog technology to growth of conifer seedlings. A project begun earlier with the Saskatchewan Wheat Pool for the development of biotechnologies for improvement of Canadian wheat varieties continued through its second year.

Within the federal public sector, new research collaborations were begun with Agriculture Canada's Plant Research Centre (Ottawa, Ont) for cryopreservation of plant germplasm, and with Agriculture Canada (Saskatoon, Sask) for investigation of the molecular biology of blackleg disease in *Brassica* species. Numerous smaller scientific collaborations with the Department also proceeded during the year. Methods for micropropagation of western white pine was a collaboration with Forestry Canada (Victoria, BC).

Several collaborative projects were concluded. These included work on plant growth promotion by bacteria, conducted with Allelix Agriculture Inc (Mississauga, Ont), evaluation of hardware for cell electroporation (New Age

Devices, Cambridge, Ont.), search for and characterization of wheat plants resistant to wheat midge attack (Saskatchewan Wheat Pool, Regina, Sask.), and a brief study of a plant virus of interest to Agriculture Canada (Vancouver, BC).

6.2.3 Services to Canadian Research

Services provided for fees to recover costs included mass spectrometric sample analyses, nuclear magnetic resonance sample analyses, custom syntheses of oligonucleotides, rental of equipment and space on our premises, and provision of research materials to on-site guests.

Scientific-technical service is also an aspect of PBI's core program. Nearly ten thousand hours of staff time were devoted to providing scientific services without fees, and the majority of this time came from Ph.D.-level research personnel. The services included:

- Biotechnology methods training in our laboratories, for industrial and international guests;

- Supervision and advice for graduate students performing thesis research at the Institute. In all, more than two dozen students were involved of whom one-third fulfilled their degree requirements during 1988/89;

- Expert reviews and assessments of extramural proposals for research funding. The main agencies served in 1988/89 were the Natural Sciences and Engineering Research Council (NSERC), the federal Industrial Research Assistance Program (IRAP), the International Development Research Council (IDRC), the Saskatchewan Agricultural Development Fund (ADF), and the Canadian Agricultural Research Council (CARC). Other recipients of expert help from PBI were four federal departments; the Centres of Excellence program; research and development agencies in British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec and New Brunswick; and other parts of the NRC Biotechnology Program. More than 150 proposals were reviewed.

- Scientific advice and counsel via boards, committees, societies and councils. PBI staff do not hold positions in commercial organizations, and in all matters are strictly bound to respect the confidentiality of bodies advised as well as of information held by the Institute. In all these roles, Institute staff act as agents of the National Research Council and not in individual capacities;

- Refereeing of publication manuscripts. One quarter of PBI's Research Officers are members of editorial boards, and all staff scientists were referees during the year, dealing in total with more than 600 articles;

- Loans of laboratory equipment to other research groups. At year-end, eight agreements were in effect for equipment valued at more than $80 000. Changes in NRC regulations during 1988 now allow some types of unneeded apparatus to be gifted to non-profit research laboratories, but few items were so disposed;

- Provision of biological and chemical materials owned by NRC to other organizations for research use only through controlled Materials Agreements. These materials included genes, vectors, oligonucleotides, proteins, bioregulator molecules, plant and cell lines, seeds and phytochemical standards;

- Participation in national and international scientific societies, including organization and support of three major meetings on behalf of societies.

6.2.4 The National Plant Biotechnology Survey

In July 1988 the Institute began a comprehensive survey of the entire Canadian plant biotechnology community, using interviews and a questionnaire as the main tools. Questions about current and likely future technologies, research investments, areas of development and commercialization, training, commodity applications and timeframes were put to more than 600 selected participants. The results were analyzed and a 102-page report was issued at the very end of the fiscal year (J. S. Katz: *Plant Biotechnology*

in Canada: Prospects for the 1990s). This document will assist all Canadian players to plan better their activities and goals for the coming decade.

6.3 Outreach Activities

As a national laboratory, and a part of the National Research Council, the Institute maintains an array of activities whose goal is to make PBI as accessible as possible to research needs throughout Canada. Six initiatives contributed to our accessibility in 1988/89.

6.3.1 Adjunct Appointments at Universities

Half of PBI's permanent scientists hold professorial appointments at Canadian universities, mostly but not entirely at the University of Saskatchewan on whose campus the Institute is located. These facilitate close interactions between the Institute and the universities.

**Adjunct professors at the University of Saskatchewan*: Dr Fred Constabel, Dr Jan W GrootWassink and Dr W G W Kurz: Applied Microbiology and Food Science
Dr William L Crosby: Biology
Dr Sean M Hemmingsen: Microbiology
Dr Kuo-Nan Kao, Dr. John D. Mahon and Dr Gopalan Selvaraj: Crop Science and Plant Ecology
Dr Kutty K Kartha: Horticultural Science
Dr Louise M Nelson: Soil Science
Dr Suzanne Abrams: Chemistry
Dr Samuel L MacKenzie: Biochemistry

**Adjunct professor at the University of Calgary:* Dr David I Dunstan: Biology

**Adjunct professor at Simon Fraser University*: Dr Edward W Underhill: Biological Sciences

6.3.2 Affiliated Scientists

To complement adjunctships, PBI invites a limited number of outstanding extramural Canadian scientists to become Affiliated Scientists of the Institute. The current members are listed with the Institute staff and guests on page 7 of this Report.

This program got actively underway in 1988 with the first annual meeting of the Affiliates with their PBI colleagues taking place 31 August to discuss the state of plant biotechnology research in Canada and to plan a 1989 symposium.

6.3.3 Guest Researchers

The Institute accepted more than 80 qualified extramural scientists into its laboratories during the year, for collaborative or joint venture research activities. Approximately half of these were from Canadian industries, with the remainder coming from universities or from foreign research organizations -- often through the various international scientific exchange agreements maintained by Canada. The year's guests are listed on pages 7 and 8.

The National Research Council paid no salaries or stipends to these guests but did provide space, basic research materials and some laboratory services without charge. Special provision was made for guest industrial groups as these have become a prominent feature of our laboratories especially since 1986. New quarters were developed to better keep small teams together.

6.3.4 Research visits

Familiarization tours of the Institute were provided on request to thirty-three industrial groups and fifteen educational groups during the year, the purpose in each case being to make prospective users of PBI better aware of our capabilities and facilities.

Sixteen renowned plant scientists were

invited to the Institute for visits and seminars during the year, and a somewhat smaller number of other visitors also gave seminars. These visitors and their seminar topics are listed on the next page. Just over 3000 people attended these seminars.

PBI personnel in turn were invited guests and speakers at more than sixty centres during the year, in addition to their regular duties of conference attendance and business travel. This year the majority of invitations were at the international level.

Special conference events of the year at the Institute included the Second International Symposium of Secondary Metabolism of Plant Cell Cultures (4-8 July 1988) and the Fourth International Conference of the International Conifer Tissue Culture Working Group (8-11 August 1988); each was attended by more than sixty delegates from around the world.

6.3.5 Honours, Appointments, Special Assignments

Several PBI staff members held prestigious appointments or undertook new duties of special significance during the fiscal year.

Dr Suzanne Abrams: Executive Committee, Canadian Science & Technology Awareness Program
Dr Fred Constabel: Editor-in-Chief, Journal of Plant Physiology; Review Committee member for Plant Research Centre (Agriculture Canada), 1988; Review Committee member for Petawawa Forest Research Institute (Forestry Canada), 1988.
Drs William Crosby, Datla Raju and Gopalan Selvaraj: President's Fund Award for basic research, 1988/89.
Dr Vincenzo DeLuca: Extramural posting to Université de Montréal, 1988.
Dr David Dunstan: Member, International Peer Review Committee for Centres of Excellence program, 1989.
Drs Sean Hemmingsen and Edward Underhill: President's Fund Award for basic research, 1988/89.
Dr Samuel MacKenzie: PBI Co-ordinator for the NRC Protein Engineering Program, 1988.
Dr Louise Nelson: President, Canadian Society of Microbiologists.
Drs Adrian Cutler, Fawzy Georges and Mohammed Saleem: President's Fund Award for basic research, 1988/89.
Dr Warren Steck: Canadian Agricultural Research Council; Advisory Board to College of Agriculture and Member of Senate, University of Saskatchewan.

6.3.6 Outreach publications

Special events and publications were organized for PBI by Mrs Lorraine Blashill. The Institute's Annual Report for 1987/88 was published in August 1988, and four issues of the quarterly PBI Bulletin appeared during the year. These publications went to approximately 1200 selected recipients, the Bulletin largely upon their request. Printed abstracts for both of the above-mentioned conferences were prepared at PBI for dissemination to participants and to plant biotechnology centres throughout Canada.

6.3.7 PBI Seminar Series, 1988/89

1988

13 April
Dr. Michael Shuler
Cornell University, Ithaca, NY
Immobilized plant cell cultures

03 May
Ms. Debbie Summer
University of Nebraska, Lincoln, Nebraska
Conformational changes in phytochrome

09 May
Dr. Sten Stymne
Swedish University of Agricultural Sciences
Uppsala, Sweden
Biochemical models for the genetic regulation of fat quality in oil-rich seeds and how they can be developed by mutant studies

06 June
Dr. Pierre Charest
Carleton University, Ottawa, Ontario
Genetic engineering for herbicide resistance in Brassica

15 July
Dr. H.J. Jacobsen
University of Bonn, F.R.G.
Embryogenesis

25 July
Dr. B.V. Milborrow
University of New South Wales, Kensington, Australia
Abscisic acid

14 September
Dr. Aladar Szalay
Dept. Plant Sciences/ARC
University of Alberta, Edmonton, Alberta
Use of luciferase marker gene fusions for monitoring plant development

28 September
Dr. Nickolaus Weber
Bundesanstalt f. Fettforschung, Institute für Biochemie und Technologie
Münster, Fed. Rep. Germany
Preparation of biologically active ether glycerolypids using plant cell cultures

12 October
Dr. Sean Hemmingsen
Plant Biotechnology Institute (NRC)
Saskatoon, Sask.
Molecular chaperones and the post-translational compartmentation and assembly of proteins

17 October
Mr. Donald L. Byers
N.A.I.T., Edmonton, Alberta
Biotechnology Program at N.A.I.T.

26 October
Dr. Ann Oaks,
McMaster University,Hamilton, Ontario
Regulation of Nitrate reductase in higher plants: role of light and nitrate and ammonium ions

07 November
Ms. Paula Coxe
Porton Instruments Inc.,Tarzana, Calif.
Advances in protein micro-sequencing technology

09 November
Dr. Hsi-Chang Wang
University of Saskatchewan, Saskatoon, Sask.
G-banding with plant chromosomes

01 December
Dr. Suzanne Abrams
Plant Biotechnology Institute (NRC)
Saskatoon, Sask.
Chemistry and biological activity of some abscisic acid analogs

02 December
Mr. Mohan Balasubramanian
University of Saskatchewan, Saskatoon, Sask.
Chromosomal virulence genes in Agrobacterium tumefaciens

06 December
Dr. G. Selvaraj and Dr. F. Georges
Plant Biotechnology Institute (NRC)
Saskatoon, Sask.
Highlights of the conference on plant molecular biology (November 1988)

07 December
Dr. M.S. Pedras
Plant Biotechnology Institute (NRC)
Saskatoon, Sask.
Phytotoxins of Phoma lingam

1989

04 January
Dr. Kenneth Giles
Vipont Research Laboratories Inc.
Saskatoon, Sask.
and
Dr. R.T. Tyler
Plant Biotechnology Institute (NRC)
Saskatoon, Sask.
Aspects and prospects of sanguinarine production in vitro

11 January
Dr. B. Gantotti
Philom Bios, Saskatoon, Sask.
Phytopathology component of bioherbicide development

19 January
Dr. R. J. Weselake,
Plant Biotechnology Institute (NRC)
Saskatoon, Sask.
Characteristics of hydrolytic enzymes involved in seed germination.

23 January
Dr. David Taylor,
Plant Biotechnology Institute (NRC)
Saskatoon, Sask.
Studies of storage lipid biosynthesis - biochemistry for biotechnology

25 January
Dr. Allan Good
Plant Biotechnology Institute (NRC)
Saskatoon, Sask.
Co-ordinate induction of anaerobically expressed genes in barley

26 January
Dr. R. George
University of Alberta, Edmonton, Alta.
Lipid dependence of purified - ATPase from* Acholeplasma laidlawii *membranes.

30 January
Dr. G. Ferrante,
Diversified Research Inc., Toronto, Ont.
Biosynthesis of polyunsaturated fatty acids in yeast and higher plants.

02 February
Dr. S. A. Sparace,
MacDonald College, Ste. Anne De Bellevue, Que.
Fatty acid and glycerolipid biosynthesis in roots.

06 February
Dr. L. Kunst,
University of Saskatchewan, Saskatoon, Sask.
***Genetic analysis of lipid biosynthesis in* Arabidopsis thaliana**

08 February
Dr. J.P. Durkin
Division of Biological Sciences (NRC)
Ottawa, Ont.
Signal transduction pathways in animal cells: a possible model for plant cell signalling

22 February
Dr. Normand Brisson,
Universite de Montreal, Montreal, Que.
Development and stress regulation of potato tuber genes

08 March
Dr. Raju
Plant Biotechnology Institute (NRC)
Saskatoon, Sask.
Experimental systems for transient and stable gene expression in plants

22 March
Mr. Ted Szabo,
Forest Industry Development, Edmonton, Alta.
Forest Biotechnology - A Co-operative Effort in Canada.

29 March
Dr. C.L. Nessler
Texas A&M University, College Station, Texas
Molecular analysis of opium specific genes in poppy

7. Support Services

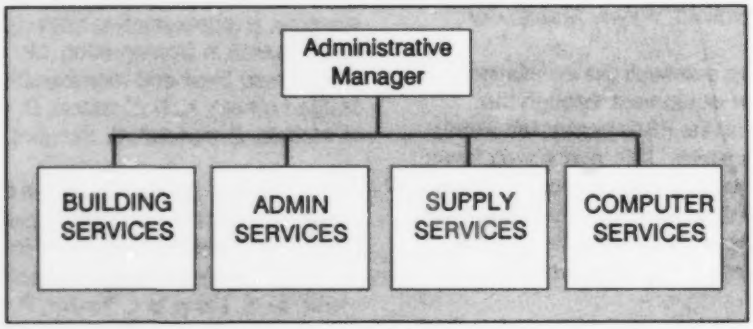
Administrative Manager : Mrs Joan L. Yorke

7.1 Overview

The Support Services section helps provide for the daily operational needs of the Institute : program administration, office services, staffing, staff training programs, building services, supply, transport, finance, research publication services and computer services.

As a publicly-owned corporation, the National Research Council is required to manage and administer its programs in accordance with the policies and standards of the Government of Canada. For example, the handling of documents must conform to the requirements of the Privacy Act, the Access to Information Act, and to many regulations. A strict accountability for funds received is also maintained. We have ongoing communications with our Ottawa headquarters. The Section provides for this, while furnishing numerous office and technical services to the research projects. The group also has responsibilities for workplace safety and for the security of the Institute.

Four units provide support services, and four of the Institute's six committees form user/provider advisory interfaces.



7.2 Service Units

7.2.1 Administrative Services

Personnel : Joan Yorke (Administrative Manager), Cheryl Bitz, Rosemarie Gallays, Ed Knapp (Research Illustration), Dawn Larson, Kathryn Seabrook, Kathy Wilson

During 1988/89 we replaced our Telex system with a facsimile station, and as a result of policy and program changes throughout the National Research Council, we re-organized internal duties to provide larger financial and personnel services. No staff changes occurred.

7.2.2 Building Services

Personnel : T. M. Mallard (Superintendent of Services), Bill Brumwell, Armand Fendelet, Rick McDonald, George Reynolds.

Building Services and Computer Services were established as separate units in 1988/89 following many years together as "general services". Building Services carried out major renovations of two laboratories during the year and installed additional new energy-saving systems on the premises. Some new space for guest researchers was also developed.

7.2.3 Computer Services

Personnel : T. M. Mallard (Superintendent of Services), Harry Holfeld, Wayne Stankowich.

This group oversees the installation and repair of computer equipment through the Institute, and maintains PBI's central laboratory data acquisition system. This year a switchover from capital purchasing to leasing of hardware allowed a more unified approach to new systems. Microcomputer use continued to grow, and with it the needs for local networking.

7.2.4 Supply Services

Personnel : W. S. Wiley (Supply Officer), Olga Coates, Jill Horn, Irene Howe, Terry Mazurkevich, James McHarg.

This unit oversees all tendering, ordering and delivery of PBI purchases of goods, carrying out more than $2.1 million worth of transactions in 1988/89. In addition it manages the Institute's vehicles, maintains the new WHMIS (Workplace Hazardous Materials Information System) and PAMS (Physical Assets Management System) duties recently prescribed by the Council.

7.3 Committees

7.3.1 User/provider advisory groups

- **Divisional Committee on Safety and Health**. This group is part of NRC's overall organization for workplace safety and health. It advises the Administrative Manager on potential risks, and has responsibilities in hazards procedures and in first aid training for staff. Members at year-end were : M. Saleem (chair), J. Magus, J. Yorke, B. Chatson, L. Pelcher, D. Horn, S. Hemmingsen, J. Hammerlindl.

- **Computer Committee**. The group's role is to advise on future needs for computer systems; a representative from NRC's Institute for Research in Construction also sits on this committee. Year-end membership was : T. M. Mallard (chair), K. B. Chatson, D. Guenter (IRC), H. Holfeld, E. Kendall, G. Selvaraj.

- **The Library Services Committee** assists the CISTI branch in choosing book and journal orders which match current project needs in the Institute. Members were : F. Chen (CISTI, chair), M. S. Lapp, J. L. Taylor, R. T. Tyler.

- **PBI's Special Events Committee** helped organize and carry out several major events during the year including three overseas trade commission visits and two international scientific conferences. Seventeen official tour groups were shown parts of the Institute. Members at year end were L. Blashill (chair), J. Condie, L. Hoffman and L. Steinhauer.

7.3.2 Research Program Committees

Research program committees exist at PBI to facilitate inter-project planning, financing, contracting-out and in general the co-ordination and evaluation of the whole research program.

- **The Planning and Review Committee** is the Institute's principal management committee where managers and project leaders meet to advise the Director and on some matters to take action collegially. The group has major responsibilities in program planning and in annual assessments of project performance. Members for the year were : W. F. Steck (chair), F. Constabel, W. L. Crosby, D. I. Dunstan, K.K.Kartha, W.G.W. Kurz, J. D. Mahon, E. W. Underhill, C. Walker (secretary) and J. L. Yorke.

- **The Plant Biotechnology Fund Committee** assessesd contracting-out proposals for 1989/90 and reviewed contract results from last year. Through changed procedures, the assessment process was streamlined in 1988/89. Members were : W. G. W. Kurz (chair), F. Constabel, W.L. Crosby, D. I. Dunstan, K. K. Kartha, J. S. Katz (secretary), J. D. Mahon and E. W. Underhill.

8. 1988 Publications and Patents

Balsevich, J., L.R. Hogge, A.J. Berry, D.E. Games and I.C. Mylchreest. 1988. **Analysis of indole alkaloids from leaves of Catharanthus roseus by means of supercritical fluid chromatography/mass spectrometry**. Journal of Natural Products 51: 1173-1177. NRCC No. 29148.

Balsevich, J. 1988. **Monoterpene indole alkaloids from Apocynaceae other than Catharanthus roseus.** In: Cell Culture and Somatic Cell Genetics of Plants. F. Constabel and I.K. Vasil, eds., Academic Press, N.Y. Chapter 21, pp 371-384.

Bekkaoui, F., M. Pilon, E. Lainé, D.S.S. Raju, W.L. Crosby and D.I. Dunstan. 1988. **Transient gene expression in electroporated Picea glauca protoplasts**. Plant Cell Reports 7: 481-484. NRCC No. 29150.

Boyd, L., A. McHughen and G. Selvaraj. 1988. **Genetic organization of an osmotolerance function**. In: Proceedings of the Second International Congress of Plant Molecular Biology, Jerusalem, Israel. November, 1988. #525.

Carrière, Y., J.G. Millar, J.N. McNeil, D. Millar and E.W. Underhill. 1988. **Identification of a female sex pheromone in the alfalfa blotch leafminer, Agromyza frontella (Rondani) (Diptera: Agromyzidae)**. Journal of Chemical Ecology 14: 947-956.

Chalifour, F.-P. and L.M. Nelson. 1988. **Effects of continuous combined nitrogen supply on symbiotic dinitrogen fixation of faba bean and pea inoculated with different rhizobial isolates.** Canadian Journal of Botany 65: 2542-2548. NRCC No. 27515.

Chalifour, F.-P. and L.M. Nelson. 1988. **Short term effects of nitrate on nitrate reductase activity and symbiotic dinitrogen fixation in faba bean and pea.** Canadian Journal of Botany 66: 1639-1645. NRCC No. 28389.

Chalifour, F.-P. and L.M. Nelson. 1988. **Effects of time and nitrate application on nitrate reductase activity, nitrate uptake and symbiotic dinitrogen fixation in faba bean and pea.** Canadian Journal of Botany 66: 1645-1652. NRCC No. 28390.

Chanway, C.P., L.M. Nelson and F.B. Holl. 1988. **Cultivar specific growth promotion of spring wheat (Triticum aestivum L.) by co-existent Bacillus species.** Canadian Journal of Microbiology 34(7): 925-929. NRCC No. 29217.

Chen, T.H.H. and K.K. Kartha. 1988. **Cryopreservation of plant cells and organs**. In: Forest and Crop Biotechnology, Progress & Prospects. F.A. Valentine, ed., Springer Verlag, New York, Chapter 14, pp. 217-240.

Chibbar, R.N., J. Shyluk, F. Georges, C.S. Mallard and F. Constabel. 1988. **Esterase isozymes as markers of somatic embryogenesis in cultured carrot cells.** Journal of Plant Physiology. 133: 367-370. NRCC No. 29388.

Chibbar, R.N., F. Georges, J. Shyluk, C.S. Mallard, F. Constabel. **Biochemical aspects of somatic embryogenesis in cultured plant cells.** UCLA Symposium on Molecular Basis of Plant Development. Abstract: Journal of Cellular Biochemistry Suppl. 12C, 180.

Chibbar, R.N., F. Georges, J. Shyluk, C.S. Mallard and F. Constabel. 1988. **Biochemical aspects of somatic embryogenesis in cultured plant cells**. Journal of Cellular Biochemistry, Supplement 12C: 180. (UCLA Symposia on Molecular and Cellular Biology, Abstract L310, 1988).

Chriqui, D., C. David and S. Adam. 1988. **Effect on the differentiated or dedifferentiated state of tobacco with tissue on its behaviour after inoculation with Agrobacterium rhizogenes.** Plant Cell Reports 7: 111-114.

Classen, H.L., G.L. Campbell and J.W.D. GrootWassink. 1988. **Improved feeding value of Saskatchewan-grown barley for broiler chickens with dietary enzyme supplementation.** Canadian Journal of Animal Science 68: 1253.

Constabel, F. 1988. **Cytodifferentiation.** Bioflavour '87, pp. 315-325. (Walter de Gruyter & Co., Berlin). NRCC No. 27935.

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Crosby, W.L. and D.S.S. Raju. 1988. **The application of transgenic technology to plant viral protection --present and future strategies.** In: Report of the Plant Virus-Biotechnology Meeting, Agriculture Canada, Vancouver, August, 1988.

Cutler, A.J. and M. Saleem. 1988. **Use of 2', 7'-dichlorofluorescein diacetate to monitor peroxidase activity in protoplasts.** Abstract: Plant Physiology 86: 152S.

Dawson, P.S.S. 1988. **The "average cell" - a handicap in bioscience and biotechnology.** Trends in Biotechnology 6: 87-90. NRCC No. 28865.

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Dunstan, D.I. 1988. **Prospects and progress in conifer biotechnology.** Canadian Journal of Forest Research 18(12): 1497-1506. NRCC No. 29145.

Dunstan, D.I. **Forest biotechnology: tissue culture for tree improvement.** Canadian Pacific - CAPE Symposium, McGill University, September 26, 1988, pp 1-22.

Georges, F., S. Girard, R.N. Chibbar and F. Constabel. 1988. **Enhancing the sensitivity of DNA detection and recovery from agarose gels.** Electrophoresis 9: 213-216. NRCC No. 28386.

Good, A.G., L.E. Pelcher and W.L. Crosby. 1988. **Nucleotide sequence of a complete barley alcohol dehydrogenase 1 cDNA.** Nucleic Acids Research 16: 7182. NRCC No. 29144.

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GrootWassink, J.W.D. **Development and handling of enzyme products.** Proceedings of five International Feed Barley Workshops. The Canadian International Grains Institute and The Canadian Wheat Board, Winnipeg.

Hemmingsen, S.M., C. Woolford, S.M. van der Vies, K. Tilly, D.T. Dennis, C.P. Georgopoulos, R.W. Hendrix, R.W. and R.J. Ellis. 1988. **Homologous plant and bacterial proteins chaperone oligomeric protein assembly.** Nature 333: 330-334.

Hobbs, S.L.A. 1988. **Genetic variability in photosynthesis and other leaf characters in Brassica.** Photosynthetica 22: 388-393. NRCC No. 29146.

Hogge, L.R., D.W. Reed and E.W. Underhill. 1988. **The identification of desulfoglucosinolates using thermospray liquid chromatography/mass spectrometry.** Journal of Chromatographic Science 26: 348-351. NRCC No. 28871.

Hogge, L.R., D.W. Reed, E.W. Underhill and G.W. Haughn. 1988. **HPLC separation of glucosinolates from leaves and seeds of Arabidopsis thaliana and their identification using thermospray liquid chromatography - mass spectrometry.** Journal of Chromatographic Science 26: 551-556. NRCC No. 29493.

Hussain, A.A.K., C. Barber and G. Selvaraj. 1988. **Chitinase enzymes in Brassica napus L. cv. Westar.** In: Proceedings of the Second International congress of Plant Molecular Biology, Jerusalem, Israel. November, 1988. #540.

Iacomini, M., P.A.J. Gorin and M. Baron. 1988. **Novel D-Glucans obtained by dimethyl sulfoxide extraction of the lichens Letharia vulpina, Actinogyra muehlenbergii, and an Usnea sp.** Carbohydrate Research 176: 117-126. NRCC No. 24766.

Jackson, J.A. and P.J. Dale. 1988. **Callus induction, plant regeneration and an assessment of cytological variation in regenerated plants of Lolium multiflorum L.** Journal of Plant Physiology 132: 351-355.

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Kalmakoff, M.L., M.D. Pickard and J.W.D. GrootWassink. 1988. **Resistance of green pigments in commercial canola oil to enzymatic hydrolysis.** Canadian

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Kao, K.N. 1988. **In vitro culture in barley breeding.** In: IAEA-TECDOC. 455: 183-191.

Kartha, K.K., L.C. Fowke, N.L. Leung, K.L. Caswell and I. Hakman. 1988. **Induction of somatic embryos and plantlets from cryopreserved cell cultures of white spruce (Picea glauca).** Journal of Plant Physiology 132: 529-539. NRCC No. 28700.

Khouri, H.E., V. De Luca and R.K. Ibrahim. 1988. **Enzymatic synthesis of polymethylated flavonols in S-adenosyl-L-methionine: 3-methylquercetin-7-0-methyltransferase.** Archives of Biochemistry and Biophysics 265: 1-7.

Kurz, W.G.W. 1988. **Semicontinuous metabolite production through repeated elicitation of plant cell cultures - A novel process.** Symposium proceedings "Plant Biotechnology: Research Bottlenecks for Commercialization and Beyond", Chapter 5, pp. 93-103. (ed. Tom J. Mabry), The University of Texas at Austin, U.S.A., April 6-7, 1987. NRCC No. 26782.

Kurz, W.G.W., R.T. Tyler and I. Roewer. 1988. **Elicitation - a method to induce metabolite production by plant cell cultures.** Proceedings of the VIIIth International Biotechnology Symposium, July 17-22, 1988, Paris, France. pp. 193-204. NRCC No. 28874.

Lapp, M.S. and J. Malinek. 1988. **Micropropagation of western white pine.** Western White Pine Management Symposium, Nakusp, British Columbia, May 2-5, 1988, pp 39-41. NRCC No. 29149.

Lazar, M.D., T.H.H. Chen, L.V. Gusta and K.K. Kartha. 1988. **Somaclonal variation for freezing tolerance in a population derived from norstar winter wheat.** Theoretical Applied Genetics 75: 480-484. NRCC No. 28387.

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McAfee, B.J. and J.A. Fortin. 1988. **Indigenous ectomycorrhizal colonization on outplanted black spruce and jack pine seedlings in reforestation sites in Quebec.** Canadian Workshop on Mycorrihizae in Forestry (M. Lalonde and Y. Piché, eds.), pp 135-136.

McAfee, B.J. and J.A. Fortin. 1988. **Comparative effects of the soil microflora on ectomycorrhizal inoculation of conifer seedlings.** New Phytologist 108: 443-449.

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Pivnick, K.A., D.L. Barton, J.G. Millar and E.W. Underhill. 1988. **Improved pheromone trap exclusion of the bruce spanworm Operophtera bruceata (Hulst) (Lepidoptera: Geometridae) when monitoring winter moth Operophtera brumata (L.) populations.** Canadian Entomologist 120: 389-396. NRCC No. 28702.

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1988 POSTERS/PRESENTATIONS

Abrams, S.R. **Abscisic acid analogs - green box chemistry.** Department of Psychiatric Research, University of Saskatchewan, Saskatoon, February, 1988.

Abrams, S.R. **Abscisic acid analogs - structure and activity.** The Third Chemical Congress of North America. Toronto, Ontario, June, 1988.

Abrams, S.R. **Chemical characterization of metabolites of ABA produced from brome grass suspension cultures.** 13th International Plant Growth Substances Conference. Calgary, July 1988.

Abrams, S.R. **Chemistry and biological activity of ABA analogs.** Department of Chemistry, University of Saskatchewan, Saskatoon, September, 1988.

Abrams, S.R. **Chemistry and biological activity of abscisic acid analogs.** PBI, Saskatoon, November, 1988.

Baillie, A.M.R., B.G. Rossnagel and K.K. Kartha. **Tissue culture regeneration and In vitro regeneration in barley.** Agriculture Institute of Canada, National Conference, Calgary, Alberta, August 21-24, 1988.

Balsevich, J. and G. Bishop. **Distribution of catharanthine, vindoline and 3',4'-anhydrovinblastine in the aerial parts of some Catharanthus roseus plants and the significance thereof in relation to alkaloid production in cultured cells.** Second International Symposium on Primary and Secondary Metabolism of Plant Cell Cultures, Saskatoon, Saskatchewan, July 1988.

Bekkaoui, F. and D.I. Dunstan. **Permeabilization of Picea glauca protoplasts using polyethylene glycol and electroporation.** 2nd Canadian Plant Tissue Culture and Genetic Engineering Workshop, Ottawa, Ontario, May 28 - June 2, 1988.

Chanway, C.P., L.M. Nelson and F.B. Holl. **Cultivar-specific growth promotion of spring wheat by co-existent Bacillus species under gnotobiotic conditions.** Annual Meeting of Canadian Society of Microbiologists, Windsor, Ontario, June 19-23, 1988.

Crosby, W.L. **The application of transgenic technology to crop improvement.** Invited Presentation to the Canadian Association of Biological Safety, Calgary, June 1988.

Davin, L., G. Haughn, D. Reed, M. Giblin and E. Underhill. **Biochemical genetics of glucosinolates in Arabidopsis thaliana.** Phytochemical Society of North America, Iowa City, June 26-30, 1988.

De Luca, V., N. Brisson, J. Balsevich and W.G.W. Kurz. **Regulation of vindoline biosynthesis in Catharanthus roseus: molecular cloning of the first and last steps in biosynthesis.** Second International Symposium on Primary and Secondary Metabolism of Plant Cell Cultures, Saskatoon, Saskatchewan, July 1988.

De Luca, V. **Cloning of genes involved in indole alkaloid biosynthesis in Catharanthus roseus.** Invited presentation, 2nd International Symposium on Primary and Secondary Metabolism in Plant Cell Cultures. Saskatoon, Saskatchewan, July 1988.

De Luca, V. **Characterization of enzymes of the late stages in vindoline biosynthesis in Catharanthus roseus.** Department of Botany, University of Toronto, April 1988.

De Luca, V. **Regulation of indole alkaloid biosynthesis in Catharanthus roseus.** Second Canadian Plant Tissue Culture and Genetic Engineering Workshop, Ottawa, Ontario, May 28, 1988.

DeYoung, R.M., R.J. Copeman, A.M. Muehlchen, M.S. Lapp and D.I. Dunstan. **Erwinia spp. cause galls on Douglas fir in coastal British Columbia.** Canadian Phytopathological Society Meeting, Vancouver, British Columbia, August 13 - 17, 1988.

Dunstan, D.I., F. Bekkaoui, F. Pilon, L.C. Fowke and S. Abrams. **Effects of ABA and analogues of Picea glauca somatic embryos.** 2nd Canadian Plant Tissue Culture and Genetic Engineering Workshop, Ottawa, Ontario, May 28 - June 2, 1988.

Dunstan, D.I., T. Bethune, S. Kikcio and M.L. Lapp. **The affect of 17-year-old Douglas fir source provenance on shoot formation in vitro.** 4th Meeting International Conifer Tissue Culture Work Group, Saskatoon, Saskatchewan, August 8 - 12, 1988.

Ellis, R.J., S.M. van der Vies, S.P. Alldrick, H.P. Lang and S.M. Hemmingsen. **Molecular chaperones: the rubisco subunit binding protein.** Agriculture and Food Research Council Discussion Meeting on Photosynthesis, Imperial College, London, April 14 -15, 1988.

Georges, F., R.N. Chibbar and F. Constabel. **New strategies in recombinant DNA technology.** Second Canadian Plant Tissue Culture and Genetic Engineering Workshop (IAPTC). p. 98, Carleton University, Ottawa, Ontario, Canada, May 29-June 2, 1988.

Georges, F. **Novel synthetic anchor oligodeoxyribonucleotides: "chem-zymatic" synthesis of artificial plant genes.** 2nd International Congress of ISPMB, Jerusalem, November 1988.

Good, A.G. and L.E. Pelcher. **Isolation and characterization of barley (Hordeum vulgare) ADH cDNA sequences.** UCLA Symposia on Molecular and Cellular Biology. The Molecular Basis of Plant Development, Steamboat Springs, Colorado, March 26 - April 2, 1988.

Good, A.G. and W.L. Crosby. **Structure and characterization of an ADH1 cDNA from H. vulgare.** UCLA Symposia on Molecular Cell Biology, Tammaron, Colorado, March 24-April 7, 1988.

GrootWassink, J.W.D., L. Nelson, L.A.K. Kolenovsky, A.D. Jain and E.W. Underhill. **Chromogenic marker for glucosinolate biosynthesis in Brassica cultures.** Genetic manipulation in plant breeding, Helsingor, Denmark, September 11 - 16, 1988.

Haughn, G.W., M. Giblin, L. Davin, L. Hogge, D. Olson and E. Underhill. **Genetic analysis of glucosinolate biosynthesis in Arabidopsis.** XVIth International Congress of Genetics, Toronto, August 21-27, 1988.

Haughn, G.W., C.R. Somerville and E. Underhill. **Arabidopsis thaliana, molecular genetics and crop improvement.** Second Canadian Plant Tissue Culture and Genetic Engineering Workshop, Ottawa, Ontario, May 29 - June 2, 1988.

Hogge, L.R. **The trials and tribulations of triglyceride analysis by GC/MS.** Western Canadian Mass Spectrometry Users Group. Saskatoon, November 14, 1988.

Hynes, R.K., C.P. Chanway, L.M. Nelson, R. Zablotowicz and J. Kloepper. **Effect of plant growth promoting rhizobacteria on growth and N_2 fixation of pea and lentil in field and laboratory conditions.** Annual Meeting of the Canadian Society of Microbiologists, Windsor, Ontario, June 19-23, 1988.

Jain, J.C., D.W. Reed, V. De Luca, J.W.D. GrootWassink and E.W. Underhill. **Assay and partial purification of two glucosinolate biosynthetic enzymes from Brassica napus.** American Society of Plant Physiologists. Reno, Nevada, July 10-14, 1988.

Kartha, K.K. and J.A. Qureshi. **Advances in wheat and barley tissue.** Second Plant Tissue Culture and Genetic Engineering Workshop (IAPTC), Carleton University, Ottawa,

May 29 - June 2, 1988.

Kartha, K.K. **Cryopreservation of an embryogenic cell culture of white spruce (Picea glauca) and subsequent induction of somatic embryos and regeneration of plantlets.** 4th International Conifer Tissue Culture Work Group, Saskatoon, Saskatchewan, August 8-12, 1988.

Kartha, K.K. **Cryopreservation of cassava tissues.** Invited talk, Workshop on Advanced Research Network for Cassava Biotechnology, CIAT, Cali, Colombia, South America, September 6-9, 1988.

Kartha, K.K. **Somaclonal variation for freezing tolerance in winter wheat.** Invited talk, Brassica Low Temperature Biotechnology Workshop, Plant Research Center, Agriculture Canada, Ottawa, March 21-22, 1988.

Kartha, K.K. Special seminar **Cryopreservation technology and applications.** University of Saskatchewan, September 26, 1988.

Kurz, W.G.W., R.T. Tyler and I.A. Roewer. **Elicitation - a method to induce metabolite production by plant cell cultures.** 8th International Biotechnology Symposium, Paris, France, July 17, 1988.

Kurz, W.G.W. **A method to induce metabolite production by plant cell cultures.** 5th Symposium of the 2nd Jacques Cartier Centre Talks, Montreal, Quebec, October 13, 1988.

Lam, K.S. and J.W.D. GrootWassink. **Enzymatic digestion of spent yeast cells for nutrient recycling in inulase production.** Society for Industrial Microbiology Annual Meeting, Chicago, Illinois, August 1988.

Lapp, M.S. **Status report on micropropagation (organogenesis and embryogenesis) from the Conifer Biotechnology Project at the Plant Biotechnology Institute.** BIOFOR III, St. John's, Newfoundland, March 24-25, 1988.

Lapp, M.S. and J. Malinek. **Effect of medium and BA concentration on production of adventitious shoots from mature embryos of western white pine.** 4th International Conifer Tissue Culture Work Group, Saskatoon, Saskatchewan, August 4-12, 1988.

MacKenzie, S.L. **Protein research at PBI and the University of Saskatchewan.** NRC Protein Fest, Division of Biological Sciences, Ottawa, June 14-15, 1988.

MacKenzie, S.L., Thomson, L. and Weselake, R. (Three presentations). **Research on B. napus liposomal and microsomal lipases.** Workshop on Lipid Modifying Enzymes. Saskatoon, September 22, 1988.

MacKenzie, S.L. **Plant Lipases - A review.** Lipase Meeting, Biotechnology Research Institute, Montreal, October 28, 1988

Martel, R., L. Pelcher and S.M. Hemmingsen. **Post-translational assembly of proteins: physiological role of heat shock proteins as molecular chaperones.** Canadian Society for Plant Molecular Biology Annual Meeting, Montreal, Quebec, August 10-12, 1988.

Newsted, W.J. **Major sclerotial polypeptides of psychrophilic fungi.** Invited talk, 5th International Congress of Plant Pathology, Kyoto, Japan, August 20-27, 1988.

Nehra, N.S., C. Stushnoff, K.K. Kartha. **Direct shoot regeneration from strawberry leaf discs.** 25th Annual Meeting of the American Society for Horticultural Science, Michigan State University, East Lansing, U.S.A., August 6-11, 1988. (Abstract in HortScience 23: 808).

Nehra, N.S., C. Stushnoff, K.K. Kartha. **Regeneration of plants from immature leaf-derived callus of strawberry.** 25th Annual Meeting of the American Society for Horticultural Science, Michigan State University, East Lansing, U.S.A. August 6-11, 1988 (Abstract in HortScience 23: 756).

Nehra, N.S., C. Stushnoff, K.K. Kartha. **Nuclear DNA and isozyme variation in leaf-induced callus cultures of strawberry (Fragaria X ananassa Duch).** XVIth International Congress of Genetics, Toronto, Canada. August 20-27, 1988 (Abstracts in Genome 30: 265).

Nelson, L.M. **Plant growth promoting rhizobacteria.** Western Branch Meeting of Canadian Society of Microbiologists, Windsor, Ontario, June 19-23, 1988.

Paiva, N.L., W.G.W. Kurz and R.T. Tyler. **Immobilization and growth of Papaver somniferum L. plant cells.** 196th National Meeting of the American Chemical Society, Los Angeles, U.S.A., September 25, 1988.

Pedras, M.S.C. **Phytotoxins from Phoma lingam.** PBI, Saskatoon, December 7, 1988.

Pilon, M., F. Bekkaoui, L.C. Fowke, S.R. Abrams and D.I. Dunstan. **Effects of ABA and analogues on somatic embryo-producing cultures of Picea glauca.** 4th Meeting International Conifer Tissue Culture Work Group, Saskatoon, Saskatchewan, August 8 - 12, 1988.

Qureshi, J.A., N. Chevrier, P. Hucl and Kartha, K.K. **Inheritance of in vitro regeneration capacity in wheat.** Proceedings of International Symposium by EUCARPIA on Genetic Manipulation in Plant Breeding - Biotechnology for the Breeder, Helsingor, Denmark, September 11 - 16, 1988.

Raju, D.S.S. and W.L. Crosby. **The application of transgenic technology to plant viral protection - present and future strategies.** In: Plant Virus Biotechnology. Agriculture Canada Research Station, Vancouver, B.C., August 1988.

Reed, D.W., L.R. Hogge, J.W.D. GrootWassink and E.W. Underhill. **Metabolic evidence for the degree of specificity of glucosinolate biosynthetic enzymes.** Phytochemical Society of North America, Iowa City, June 26-30, 1988.

Roewer, I.A., R.T. Tyler and W.G.W. Kurz. **Elicitation of benzophenanthridine alkaloids in cell suspension cultures of Papaver somniferum L.** International Symposium of the Phytochemical society of Europe, London, U.K., December 19, 1988.

Saleem, M., F. Georges, A.J. Cutler. **Flounder antifreeze protein promotes supercooling in plant tissues.** Invited talk, Brassica Low Temperature Biotechnology Workshop, Plant Research Centre, Agriculture Canada, Ottawa, March 21-22, 1988.

Sharma, S., P. Bhanot, F. Georges, B. Waygood. **Determination of residues involved in epitopes in HPr using site-directed mutagenesis.** Canadian Federation of Biological Societies 31st Annual Meeting (Abstract #227), Quebec City, Quebec, June 15-18, 1988.

Taylor, D.C. **Triacylglycerol assembly enzymes in Brassica microspore-derived embryos.** Workshop on Lipid Modifying Enzymes. Saskatoon, September 22, 1988.

Thomson, L. **Isolation and characteristics of liposomal lipase.** Lipase Meeting, Biotechnology Research Institute, Montreal, October 28, 1988

Tsang, E.W.T. **Tissue culture systems of white spruce (Picea glauca) available for agrobacterium-mediated genetic transfer and preliminary results on genetic transformation.** BIOFOR III, St. John's, Newfoundland, March 24-25, 1988.

Tsang, E.W.T., H. David, A. David and D.I. Dunstan. **Toxicity of antibiotics on zygotic embryos of white spruce (Picea glauca) cultured in vitro.** 4th Meeting International Conifer Tissue Culture Work Group, Saskatoon, Saskatchewan, August 8-12, 1988.

Tyler, R.T., C.O.M. Rijnders and W.G.W. Kurz. **Elicitation of benzophenanthridine alkaloid synthesis in poppy cell cultures.** Third Chemical Congress of North America, Toronto, Ontario, June 8, 1988.

Tyler, R.T., U. Eilert, C.O.M. Rijnders, I.A. Roewer, C.K. McNabb and W.G.W. Kurz. **Studies of benzophenanthridine alkaloid production in elicited cell cultures of Papaver somniferum L.** Second International Symposium on Primary and Secondary Metabolism of Plant Cell Cultures, Saskatoon, Saskatchewan, July 5, 1988.

Underhill, E.W. **A painting by James Gurney: Canola research at the Plant Biotechnology Institute.** Research and Technical Committee, Canola Council of Canada. Saskatoon, April 19, 1988.

Underhill, E.W. **Manipulation of glucosinolates in plant systems.** Second Canadian Plant Tissue Culture and Genetic Engineering Workshop, Ottawa, May 29 - June 2, 1988.

Wang, H., A.J. Cutler, M. Saleem, L.C. Fowke. **Preprophase bands in soybean protoplast cultures.** Gordon Research Conference: The Cytoskeleton. New Hampshire, July 1988.

Weselake, R. **Partial purification and properties of microsomal lipase from germinating seedlings of Brassica napus.** Joint Meetings of the Canadian Society of Plant Physiologists and the Canadian Botanical Association. Victoria, June 5-9, 1988.

Weselake, R. **Isolation and characteristics of microsomal lipase.** Lipase Meeting, Biotechnology Research Institute, Montreal, October 28, 1988

Wetter, L.R., Giblin, E.M. and Underhill, E.W. **Effect of media sulfate concentration on myrosinase activity and glucosinolate production and catabolism in cell suspension cultures.** Second Canadian Plant Tissue Culture and Genetic Engineering Workshop, Ottawa, May 29 - June 2, 1988.

New Patents and Applications:

S.R. Abrams and L.V. Gusta. **Abscisic Acid-Related Plant Growth Regulators-Germination Promoters.** /U.S. patent applied for December 1, 1988

J. Archambault, B. Volesky and W.G.W. Kurz. **Surface Immobilization of Plant Cells**/Australian Pat. Application No. 15054/88, April 27, 1988/U.S. Pat. Application No. 186,635, April 27, 1988/European Pat. Application No. 88.304077.6, May 5, 1988. (Great Britain, France, Germany, Italy, Sweden, Switzerland, Netherlands, Belgium)/ Japanese Pat. Application No. 116794/88, May 13, 1988.

Duck, P., R. Bender, W.L. Crosby and J.G. Robertson. **Molecules useful for the Detection of Nucleic Acid Sequences**/US Pat.# pending. Eur. Pat. # 86116906.8. Filed January 26, 1987.

Underhill, E.W., J.W. Wong, P. Palaniswamy, W.F. Steck and M.D. Chisholm. **Attractants for Fall Cankerworm Moths**/ Canadian Patent No. 1 231 983 (1988).

9. Canada's Investment at PBI 1988/89

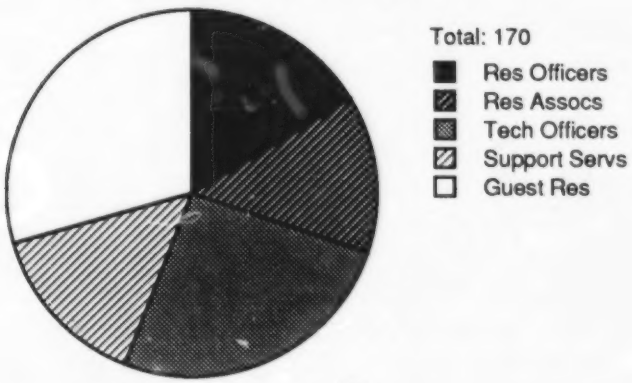
All sectors -- government, private and university -- increased their investments at PBI during the year, allowing continued delivery of a multi-sector research program by the Institute. Moreover, trends through the fiscal year suggested that the role of private sector resources will continue to grow in the years ahead.

The Government of Canada maintained 86 research positions and 24 supporting positions at PBI during the year, for a total of 110 staff of whom 26 were limited-term appointments. In addition 10 further staff were supported by special public-private funding. An average of 50 non-NRC guest researchers supported by industrial or university funds was present at PBI at any time, and an equal number of off-site people also participated in PBI project work. The distribution of workforce resources is shown in the first pie graph below, and the pattern of expenditure supported by NRC allocations is summarized in the large pie graphs on page 50.

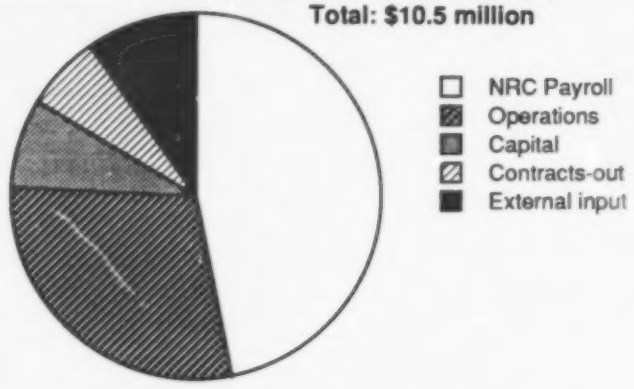
Since the establishment of the Institute in 1983, current-dollar investments have risen by nearly 25%, but PBI's purchasing power has actually declined by about 5%. Of the Institute's 1988/89 core operating allocation of $3.6 million, nearly one-third went to maintenance of buildings, facilities, heating, water and electrical services, etc., and a further $0.6 million was committed through contracts for spending at Canadian universities. The remaining $1.8 million supported all the Institute's in-house core research, business and administrative activities.

The Institute's direct cash revenues rose from $4K (1987/88) to $62K, and the value of research performed for and with industrial clients under formalized contractual agreements leapt forward from $200K to $400K. Given the limited core purchasing power base of PBI, these figures cannot show similar advances in future but will see at least modest increases in the coming year.

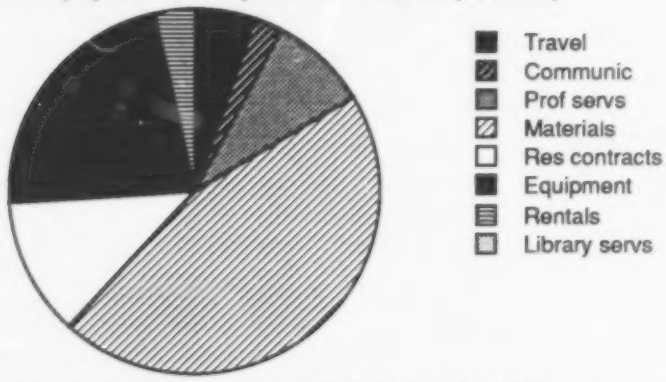
Makeup of the PBI workforce (1988/89)



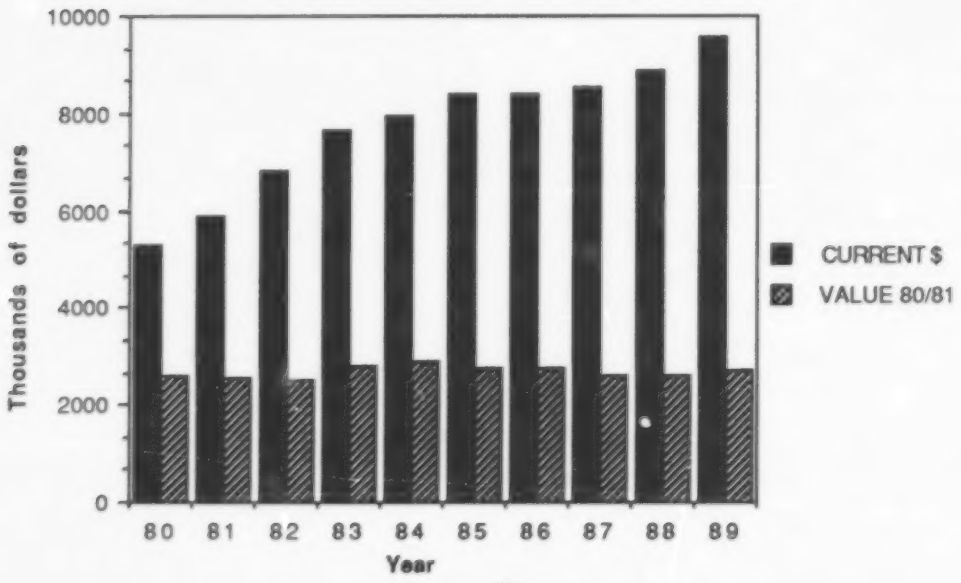
NRC and external investments at PBI (1988/89)
Total: $10.5 million



Non-payroll NRC expenditures at PBI (1988/89)



Total budgetary resources of PBI during the 1980s



The Year Ahead

The Plant Biotechnology Institute's business plan for 1989/90 calls for continued efforts in the research areas described in this report, without significant enlargements. Outputs for industrial use will continue to be PBI's highest priority.

Two urgent needs will be met in 1989/90: new greenhouse space for testing and propagation of transgenic plants; and more on-site laboratory and office units for industrial groups wishing to set up at or near the Institute. By leasing and internal renovations these facilities can be acquired to speed transfers of new technologies and products.

Thanks to improved marketing and development thrusts across NRC, the Institute's dollar value of extramural business will likely exceed $l million in 1989/90, an indication of our growing technology outputs.

Dr. Warren Steck, Director ..... (306) 975-4191
Dr. Fred Constabel, Assistant Director ..... (306) 975-5572
Mrs. Joan Yorke, Administrative Manager ..... (306) 975-4194
Dr. W.G.W. Kurz, Biotechnology Development ..... (306) 975-5570
Fax: 975-4839

110 Gymnasium Road
Saskatoon, Saskatchewan
S7N 0W9